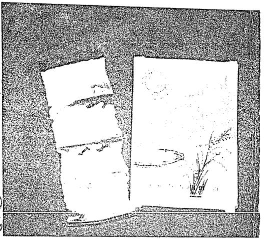
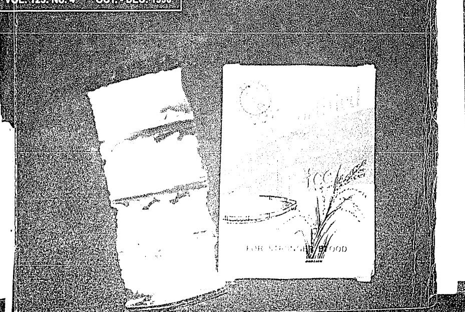
PHILIPPINE JOURNAL OF SCIENCE

VOL. 125, No. 4 OCT. - DEC. 1996





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The Philippine Journal of Science is a journal on basic sciences published quarterly by the Science and Technology Information Institute - Department of Science and Technology (STII-DOST) with editorial office in Bicutan, Taguig, Metro Manila.

OUR COVER. The cover photo, taken from the fourth manuscript published in this issue, shows sample of FVRice-Iron Fortified.

TABLE OF CONTENTS

ARAÑEZ, ADORACION, T. and RUBIO, R. O.

Genotoxicity of Pigments from Seeds of Bixa
Orellana L. (Atsuete) I.
Determined by Allium Test 259

GUEVARA, AMELIA P., APILADO, A., SAKURAI, H.,KOZUKA, M., and TOKUDA, H.

Anti-Inflammatory, Antimutagenicity,
and Antitumor-Promoting Activities
of Mahogany Seeds, *Swietenia*
Macrophylla (*Meliaceae*) 271

ARIF, MOHAMMAD and VAHIDY, A. A.

Toxicity of Fungicides Nimrod and Dithane
M-45 on Mitotic Cells of *Allium Cepa* L. 279

VALDEZ, DIVINAGRACIA H., MALLILLIN, A. C., CRUZ, E. M., KUIZON, M. D., and CABRERA, I. Z

Iron Absorption from
FVRice-Iron Fortified 291

CHAUHAN, SUSHMA., TANDON, M. P.
and BHARGAVA, V.

Annual Recurrence of *Fusaria*
in the Rhizosphere of Ornamental Crops
Grown in Different Soils of Allahabad 301

RELON, MILAGROS LONTOC

Total Mercury of Selected
Fish Species from Laguna De Bay 305

GONZAL, DOMINADOR G., DELA CRUZ L.U.
and GARRITY, DENNIS P.

Performance of *Gmelina Arborea*
with VAM Inoculation in Acid Soil 317

THE PHILIPPINE JOURNAL
OF SCIENCE

Oct. - Dec. 1996
Vol. 125 No. 4

GENOTOXICITY OF PIGMENTS FROM SEEDS OF BIXA ORELLANA L. (ATSUETE)
I. DETERMINED BY ALLIUM TEST

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ABSTRACT

Roots of Allium cepa L. (onion) were treated for 4 h with crude atsuete-chloroform extract (CE); pure petroleum ether extract (PE), and CE residue-petroleum ether extract (BE). Roots soaked in distilled water and DMSO were used as control.

Cells treated with the CE and PE exhibited significantly lower mitotic index values than those of the control. CE and PE dissolved in DMSO were genotoxic at 1.5%, 3.0%, and 4.5% (w/v) as shown by the significantly high number of mitotic aberrations in the form of anaphase bridges, ring chromosome, precocious chromosomes, and disoriented chromosomes. Mitotic aberrations and lower mitotic index values in cells exposed to BE treatments were not statistically different from those of the control.

INTRODUCTION

Bixa orellana L. (atsuete) grows as a large shrub or a small tree. The fruits are capsules with fleshy spines, round to elongated, and divided into two valves with seeds inside. The seeds are coated with a thin layer of bright yellow to red aril from which the dye is obtained. This dye is an alternative to the synthetic yellow and orange food colors used in coloring butter, margarine, cheese, ice cream and confectionery (Tong, 1984). In the Philippines, the atsuete extract is also used as food color for traditional Filipino cooking.

The U.S. Food and Drug Administration has approved around 2,700 chemical entities and plant extracts as direct food additives including natural components of foods and synthetic chemicals (Zeiger, 1993). Around 10-20% of all colorants are natural pigments (Danisova, 1989).

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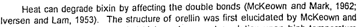
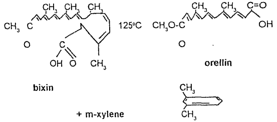
The atsuete dye contains two coloring matter, namely bixin and orellin (Tanchico and West, 1936). Bixin appears as brick red needle-like crystals, is soluble in water, ethyl alcohol, methyl alcohol and chloroform but insoluble in petroleum ether while orellin is a yellow-orange wax-like liquid, insoluble in water but soluble in ethyl alcohol, methyl alcohol, petroleum ether and chloroform (Batoon, 1978). The main component of the dye is bixin with the following structural formula (Kuhn and Winterstein, 1928)



$$H_3COOC\text{-}CH{=}CH\text{-}\overset{\overset{CH_3}{|}}{C}{=}CH\text{-}CH{=}CH\text{-}\overset{\overset{CH_3}{|}}{C}{=}CH\text{-}CH{=}CH\text{-}CH{=}\overset{\overset{CH_3}{|}}{C}\text{-}CH{=}CH\text{-}CH{=}\overset{\overset{CH_3}{|}}{C}\text{-}H{=}CHCOOH$$

The unstable *cis*-form is transformed by heat to the stable *trans*-isomer (McKeown and Mark, 1962). Bixin has a molecular weight of 394.49, is soluble in alkalis, and is precipitated by acids (Devon and Scott, 1972).

Heat can degrade bixin by affecting the double bonds (McKeown and Mark, 1962; Iversen and Lam, 1953). The structure of orellin was first elucidated by McKeown and Mark (1962). They proposed that bixin undergoes degradation upon high temperature extraction to form a yellow pigment, presumed to be orellin, which is the monomethyl ester of the unsymmetrical tetradecahexane-dioic acid. Degradation of bixin as proposed is given below:



The above result showing that the yellow dye is a degradation product of bixin was confirmed later (Osteraas and Olsen, 1967).

Thin layer chromatography of chloroform extract produced five spots (Tong, 1984). One major spot could be cis-bixin, one minor spot could be orellin, while the other spots are likely to be the degradation products of cis-bixin.

The growing concern over some dangers posed by natural food-based additives has prompted the reevaluation of these products for their toxicity and ability to cause mutations in some test organisms.

Studies on the mutagenicity of pigments from seeds of atsuete were done by Banzon and Arañez (1984) by using the Ames Test, Micronucleus Test and *Drosophila* Test. The crude extract and petroleum ether extract were observed to produce a significant increase in the micronucleated polychromatic erythrocytes of mice and induced significant increases in the number of flies carrying wing abnormalities. In the bacterial test using *Salmonella typhimurium* TA 1535, TA 1537, TA 98, and TA 100 no significant increase in the number of revertant colonies in the materials tested was observed. Inasmuch as there was no microsomal enzyme used in the bacterial test, it was concluded that the mutagenic agent was probably a promutagen.

Chromosomal aberrations constitute a reliable, effective, and economical bioassay for environmental pollutants (Grant, 1978). Higher plants have been proposed as test organisms for the detection of genotoxic substances in the environment (Rank and Nielsen, 1993). Among the test systems, using *Allium cepa* chromosomes has been found to be a good method due to the large size of the chromosomes and their sensitivity to environmental changes (Grant, 1982). Plant test systems using *Vicia faba* and *Allium cepa* showed extremely good correlation with the bacterial and mammalian test systems (De Kergommeaux et al., 1983). The Allium assay was also proposed as a standard method for the testing of chemicals, environmental monitoring and toxicity screening of waste water and river water (Fiskesjo, 1985).

The objectives of the study are (1) to determine the effects of the crude atsuete-chloroform extract (CE), the CE residue-petroleum ether extract (BE) and the pure petroleum ether extract (PE) on the mitotic index of cells from the root meristem of *Allium cepa L.*; and (2) to determine the different kinds and frequencies of mitotic aberrations produced by the agents in the root meristem of *A. cepa.*

MATERIALS AND METHODS

Extraction of Pigments

Ripe fruits of Bixa orellana L. (atsuete) collected at the Diliman campus of the University of the Philippines were used in this study.

The crude extract was prepared by using 1 L chloroform (AR grade) for every 500 g of dried atsuete seeds. The seeds in chloroform were boiled for 1 h using a water bath. The liquid extract was poured off from the seeds, then the chloroform was evaporated by using a rotary evaporator (Buchi 461) and a drying oven at a temperature of around 65°C. The weight of the crude atsuete-chloroform extract (CE) was determined. A portion of the CE to be used in the test was set aside.

The remaining CE was treated with petroleum ether (AR grade). Several changes of petroleum ether were made until the petroleum ether was already colorless. The residue after extraction with petroleum ether was dried in an oven and designated as BE. BE was subjected to TLC using silica gel F 254 (Merck). The flowing solvent used was glacial acetic acid and hexane (1:5, v/v). Spotting was done using 10 µL of BE.

The petroleum ether from the extract was evaporated by using a rotary evaporator and a drying oven at a temperature of around 65°C leaving behind an oily dark red pigment designated as PE. The weight of PE was determined. PE was subjected to TLC following the same procedure used for BE except that petroleum ether was used as solvent instead of chloroform.

Allium Test

Onion bulbs of more or less equal sizes were germinated in separate vials filled with distilled water. Their outer scales were peeled off to expose the root primordia. Bulbs with many roots were selected for the experiment.

Varying concentrations of CE, BE, and PE were prepared in different vials using dimethyl sulfoxide (DMSO) as solvent. Based on a preliminary study, the doses used for the three different agents were 1.5%, 3.0%, and 4.5% (w/v). Onion roots soaked in distilled water and DMSO were used as control.

Three onion bulbs for each dose were used when the roots have grown to about 1 cm in length. The onion bulbs were placed in their respective treatment vials with their roots soaked in the treatment solution. The entire set-up was kept in the dark for 4 h.

The treated roots were washed with tap water for 2 h immediately after the treatment. Afterwards they were transferred to other vials containing distilled water for another 24 h. The roots were then excised, killed and fixed in freshly prepared Farmers solution which is composed of alcohol and glacial acetic acid (3:1). The root tips were stored overnight in the refrigerator. Root tips that were not studied immediately were transferred to 70% alcohol at 4°C for prolonged storage.

In preparing root tips for mitotic studies, 1-3 drops of 1 N HCl were added to each root tip on a slide. After about 5 min the HCl was drained followed by staining with 1% acetocarmine. The slide was passed over a flame 5-10 times. A cover slip was placed over the specimen, then squashed and sealed.

A total of 30 slides was prepared for each dose. One hundred cells per slide were observed for the determination of mitotic index, computed as the number of dividing cells over the total number of cells observed multiplied by 100, and the kind and frequency of mitotic aberrations. Photomicrographs of cells with abnormal mitosis were taken.

Analysis of variance, Duncans multiple range test, and Least significant difference test were used in the analysis of data.

RESULTS

Extraction of Pigments

CE, BE, and PE appeared as brick-red sticky solid, brick-red powder, and yellow orange wax-like liquid, respectively. When BE was subjected to thin layer chromatography, only one spot was observed with an Rf value of 0.47. When PE was subjected to the same test five spots with Rf values of 0.90, 0.63, 0.56, 0.16 and 0.09 were detected. For every 100 g of atsuete seeds, 540 mg of CE, 320 mg of BE, and 180 mg of PE were obtained.

Allium Test

Crude Atsuete-Chloroform Extract (CE)

The CE induced a decrease in the mitotic index of cells from the treated onion meristems. The mitotic index values of onion root meristems treated with 1.5%, 3.0%, and 4.5% CE in DMSO were 11.73, 12.37, and 14.73, respectively. Those soaked in distilled water and DMSO have mitotic index values of 16.37 and 17.20, respectively. The mean mitotic index values of the different treatments are in Table 1. The mitotic index values of cells treated with 1.5% and 3.0% were found to be significantly different from those soaked in distilled water and DMSO.

Varying doses of CE produced mitotic aberrations in the cells from treated root meristems of onion. The most predominant aberrations observed were single and multiple bridges (Figure 1). Ring, precocious and disoriented chromosomes (Figures 2-3) were also observed. Mitotic aberrations in onion root tip cells treated with 1.5%, 3.0%, and 4.5% CE in DMSO were 7.67%, 5.66%, and 7.01%, respectively (Table 2). The aberrations in the control were significantly lower than the aberrations in root tip cells treated with CE in DMSO.

CE Residue-Petroleum Ether Extract (BE)

Cells treated with 1.5%, 3.0%, and 4.5% BE in DMSO had mitotic index values of 12.87, 12.97, and 14.37, respectively. The cells soaked in distilled water and DMSO had mitotic index values of 16.37 and 17.20, respectively, which were higher than those treated with BE. However, the differences in the mitotic index values of cells from the control and those treated with BE were not significant (Table 3).

The presence of aberrations was noted in cells treated with BE in DMSO (Table 4). Cells from root meristems soaked in 1.5%, 3.0%, and 4.5% BE in DMSO had 0.78%, 1.55%, and 1.39% aberrations, respectively. Those soaked in distilled water and DMSO had 0.82% and 3.10%, respectively. Analysis of variance revealed no significant differences in the frequency of mitotic aberrations between cells from the treated meristems and from the control.

Pure Petroleum Ether Extract (PE)

A decrease in the mitotic index values was observed in root meristems treated with 1.5%, 3.0%, and 4.5% PE in DMSO as compared with those in distilled water and DMSO (Table 5). Mitotic index values of root meristems treated with 1.5%, 3.0%, and 4.5% PE were 14.77, 11.57, and 12.83, respectively. Those soaked in distilled water and DMSO had mitotic index values of 16.37 and 17.20, respectively. Only the mitotic index value of root meristems treated with 3.0% PE was noted to be significantly different from that of the control.

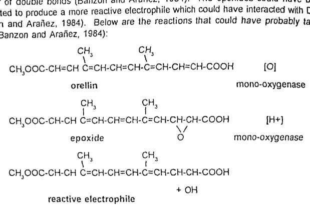
Percent mitotic aberrations in the form of single and multiple bridges and observed in cells treated with 1.5%, 3.0%, and 4.5% PE in DMSO were 7.90%, 10.09%, and 10.08%, respectively. For cells soaked in distilled water and DMSO, the percentages of mitotic aberrations were 0.82% and 3.10%, respectively (Table 6). Differences in the frequency of aberrations between cells treated with varying concentrations of PE and those of the control were significant.

DISCUSSIONS

In the present experiment, the mitotic index values of cells treated with CE decreased with a corresponding increase in dosage. However, in the case of PE, only a slight inhibition was observed when the dose was increased. BE also inhibited mitotic division in treated cells, although this was found to be not significant when compared with the control. In general, inhibition of mitotic division has been demonstrated with the different doses of CE, BE, and PE.

The CE and PE were found to cause significantly higher number of mitotic aberrations in *A. cepa.* The genotoxic agent in CE and PE is probably a promutagen. In the bacterial test system with no microsomal enzyme used, a negative result for the mutagenicity of the above agents was observed while a positive result was obtained in the Micronucleus Test (Banzon and Arañez, 1984).

The genotoxic agent in CE and PE may be orellin. Based on some studies, there are two pigments in the atsuete dye, bixin and orellin (Tanchico and West, 1936; Batoon, 1978). Orellin is soluble in petroleum ether unlike bixin (Batoon, 1978). However, degradation products from bixin (Tong, 1984) and other organic compounds may also be present in PE which may be genotoxic. If orellin is the genotoxic agent in PE, then orellin could have been converted into an epoxide by undergoing oxygen insertion at a number of double bonds (Banzon and Arañez, 1984). The epoxides could have been protonated to produce a more reactive electrophile which could have interacted with DNA (Banzon and Arañez, 1984). Below are the reactions that could have probably taken place (Banzon and Arañez, 1984):



$$CH_3OOC\text{-}CH{=}CH\ \overset{CH_3}{C}{=}CH\text{-}CH{=}CH\text{-}\overset{CH_3}{C}{=}CH\text{-}CH{=}CH\text{-}COOH \qquad [O]$$

orellin mono-oxygenase

$$CH_3OOC\text{-}CH\text{-}CH\ \overset{CH_3}{C}{=}CH\text{-}CH{=}CH\text{-}\overset{CH_3}{C}{=}CH\text{-}\underset{O}{CH\text{-}CH}\text{-}COOH \qquad [H+]$$

epoxide mono-oxygenase

$$CH_3OOC\text{-}CH\text{-}CH\ \overset{CH_3}{C}{=}CH\text{-}CH{=}CH\text{-}\overset{CH_3}{C}{=}CH\text{-}CH\text{-}CH\text{-}COOH$$

$+\ OH$

reactive electrophile

The above reactions are made possible by the presence of a microsomal enzyme system in mice, the experimental animals used in the Micronucleus Test. In the present work, which made used of meristematic cells of onion, an enzyme system similar to the microsomal enzyrne system found in mice may be present.

The epoxide could act as alkylating agents (Lim-Sylianco, 1987). Alkylated guanine may be released from the DNA thus producing apurinic sites (Scalera and Ward, 1971) which may lead to strand brakes (Manson, 1980). When double strand breaks are produced chromosome fragmentation may then occur. The broken ends of chromosomes may eventually unite forming ring chromosomes. Fragments from two different chromosomes with a centromere each may unite forming a dicentric chromosome. The dicentric chromosome could also give rise to chromosome bridges.

In another study (Banzon and Arañez, 1984), the Micronucleus Test gave a positive result for the mutagenicity of the crude extract and petroleum ether extract and a negative result from the residue left when the crude extract was treated with petroleum ether (which is designated as BE in the present study). The above results are in agreement with the results of the present study. The Micronucleus Test like the Allium Test can detect the clastogenic effect of an agent. Point mutations in the form of base substitution and frameshift mutation cannot be detected by the Allium Test. Other tests are needed to determine the genotoxicity of BE.

A follow-up study using Dominant Lethal Test is being conducted to determine the genotoxicity of BE. Another test using sister chromatid exchange on pure bixin and orellin will be made soon. It is recommended that in addition to sister chromatid exchange other test systems be used on pure bixin and orellin.

ACKNOWLEDGMENT

The authors would like to thank the Natural Sciences Research Institute for the financial support, Miss Maria Etheliori A. Mariano and Miss Mary Beth I. Bacano for the technical assistance.

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Table 1. Mitotic index values of *Allium* cepa root tip cells treated with crude atsuete-chloroform extract (CE).

Dose (w/v)	Total No. of Cells	No. of Mitotic Cells	Mitotic Index*
0 (distilled water)	3,000	491	16.37a
0 (DMSO)	3,000	516	17.20a
1.5	3,000	352	11.73b
3.0	3,000	371	12.37b
4.5	3,000	442	14.73ab

*Mitotic index values followed by the same letters do not differ significantly at 0.05 level (Duncan's Multiple Range Test).

Table 2. Frequency of chromosome aberrations in *Allium* cepa root tip cells treated with crude atsuete-chloroform extract (CE)

Dose (w/v)	Total No. of Cells	No. of Mitotic Cells	No. of Cells With Aberrations	% Aberration
0 (distilled water)	3,000	491	4	0.82
0 (DMSO)	3,000	516	16	3.10
1.5	3,000	352	27	7.67*
3.0	3,000	371	21	5.66ns
4.5	3,000	442	31	7.01*

**Significant at 0.05 level

nsNot significant at 0.05 level

Table 3. Mitotic index values of *Allium* cepa root tip cells treated with CE residue petroleum ether extract (BE)

Dose (w/v)	Total No. of Cells	No. of Mitotic Cells	Mitotic Index*
0 (distilled water)	3,000	491	16.37a
0 (DMSO)	3,000	516	17.20a
1.5	3,000	386	12.87a
3.0	3,000	389	12.97a
4.5	3,000	431	14.73a

*Mitotic index values followed by the same letters do not differ significantly at 0.05 level (Duncan's Multiple Range Test).

Table 4. Frequency of chromosome aberrations in *Allium* cepa root tip cells treated with crude atsuete-chloroform extract (CE).

Dose (w/v)	Total No. of Cells	No. of Mitotic Cells	No. of Cells With Aberrations	% Aberration
0 (distilled water)	3,000	491	4	0.82
0 (DMSO)	3,000	516	16	3.10
1.5	3,000	386	3	0.78^{ns}
3.0	3,000	388	6	1.55^{ns}
4.5	3,000	431	6	1.39^{ns}

^{ns}Not significant at 0.05 level

Table 5. Mitotic index values of Allium cepa root tip cells treated with pure petroleum ether (PE).

Dose (w/v)	Total No. of Cells	No. of Mitotic Cells	Mitotic Index*
0 (distilled water)	3,000	491	16.37ab
0 (DMSO)	3,000	516	17.20a
1.5	3,000	443	14.77abc
3.0	3,000	347	11.57c
4.5	3,000	385	12.83bc

*Mitotic index values followed by the same letters do not differ significantly at 0.05 level (Duncan's Multiple Range Test).

Table 6. Frequency of chromosome aberrations in *Allium* cepa root tip cells treated with pure petroleum ether extract (PE).

Dose (w/v)	Total No. of Cells	No. of Mitotic Cells	No. of Cells With Aberrations	% Aberration
0 (distilled water)	3,000	491	4	0.82
0 (DMSO)	3,000	516	16	3.10
1.5	3,000	443	35	7.90*
3.0	3,000	347	35	10.09*
4.5	3,000	387	39	10.08*

*Significant at 0.05 level

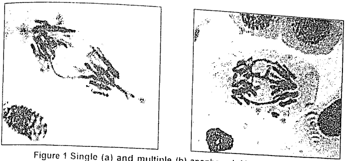

Figure 1 Single (a) and multiple (b) anaphase bridges in *A. cepa* treated with CE, x1389.

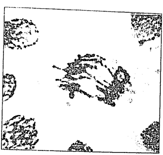

Figure 2 Multiple anaphase bridges and ring chromosome in *A. cepa* cell treated with CE, x1389.

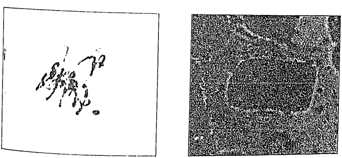

Figure 3 Precocious (a) and disoriented (b) chromosomes in *A. cepa* cell treated with CE, x1389.

ANTI-INFLAMATORY, ANTIMUTAGENICITY, AND ANTITUMOR-PROMOTING ACTIVITIES OF MAHOGANY SEEDS, SWIETENIA MACROPHYLLA (MELIACEAE)

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ABSTRACT

The crude ethanol extract of mahogany (Swietenia macrophylla King) seeds was tested for anti-inflammatory, antimutagenic, and antitumor-promoting activities. Solvent fractions of the ethanol extract, namely hexane, CCl_4 and methanol fractions were also tested for their anti-inflammatory and antitumor-promoting activities.

At a dosage of 1 mg per gram body weight of the test mice, the ethanol extract reduced carrageenan-induced inflammation by 79%. Less activities were observed for the solvent fractions, with the highest inhibition given only at 60% for the methanol fraction and a low of 23 % inhibition for the hexane fraction. These observations indicate anti-inflammatory activity.

Mutagenicity/antimutagenicity of the ethanol extract was studied using the Micronucleus test. The results indicate antimutagenicity of the crude extract. At a dosage of 0.02 mg per gram body weight, the extract reduced the number of micronucleated polychromatic erythrocytes induced by mitomycin C, a known mutagen, by almost 50%.

The antitumor promoting activity of the crude extract and the solvent fractions were studied using the Epstein-Barr virus early-antigen (EBV-EA) activation, with 12-0-tetradecanoylphorbol-13-acetate (TPA) as the tumor promoter. The results indicate significant inhibitory effects on EBV-EA activation indicating the antitumor- promoting activity.

INTRODUCTION

New plants are being tapped as "cure-all medicine" for common illnesses, like arthritis, rheumatism, gout, diabetes, fever, cough, and high blood pressure. One of the more recent choices and now becoming a fad is mahogany (Swietenia macrophylla King.) seeds. The seeds are dehulled and simply taken in like a pill.

A member of the Meliaceae, this tree was introduced from Central America as a source of hardwood. It can reach 20 m high and is distinguished by its straight trunk and compounded dark green leaflets, 12 cm long and 6 cm wide, 3 to 6 pairs of which comprise a leaf (Madulid, 1995). The tree is sought after for its shade and its reddish brown wood.

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The bark of the closely-related species Swietenia mahagoni (L.) Jacquin is used in the West Indies as a cinchona (antimalarial) substitute. It is also used as an antipyretic, a tonic, and as an astringent (Quisumbing, 1978).

This study reports the bioactivities of the crude ethanol extract of the dried seeds of mahogany and its solvent fractions particularly its anti-inflammatory, antimutagenic, and antitumor-promoting activities.

MATERIALS AND METHODS

Extraction

Mahogany seeds were sourced from Pangasinan, Laguna and Zamboanga. After dehulling, the seeds were ground with a blender and soaked in distilled ethanol for 2 d with occasional stirring. This alcoholic extraction was done three times. The resulting extract was filtered and then concentrated under reduced pressure using a rotary evaporator set at 40°C. A portion of the crude ethanol extract was successively partitioned in hexane, carbon tetrachloride, and methanol (Apilado, 1996).

The crude ethanol extract and the solvent fractions were bioassayed for their anti-inflammatory, antitumor -promoting, and antimutagenic activities.

Bioassay for Anti-inflammatory Activity: Carrageenan-induced edema method (Winter, et al. 1962; Espino, 1994)

The anti-inflammatory activities of the extracts were tested using the carrageenan-induced edema method using Swiss Webster mice as test animals. Five mice per test group were used.

The mice, each weighing about 20 g, were fasted for 16 h prior to the experiment proper. After fasting, the initial volume of the right hind paw was measured using a fabricated plethysmometer. The test extracts, dissolved in 2% carboxymethylcellulose (CMC), were administered orally with a feeding gavage at a dosage of 1 mg/g body weight. After 1 h, 0.05 ml of a 2% carrageenan-saline solution was injected intradermally into the right hind paw of the mice. The volume of the right hand paw was again measured 3 h after injection. The % inhibition of inflammation of the test groups were compared with the control group which was treated with 2% CMC and carrageenan. Indomethacin, a well known anti-inflammagen, was used as a positive control, given orally by gavage, at a dosage of 0.01 mg/g body weight.

Percentage inhibition of inflammation was calculated as:

% Inhibition = $[(A-B)/A] \times 100$, where

A is the paw volume of the mice treated with CMC (negative control)
B is the paw volume of the mice treated with extract or solvent fraction or indomethacin (positive control)

Bioassay for Mutagenic/antimutagenic activity: the micronucleus test (Schmid, 1974)

The mutagenic/antimutagenic activity of the crude ethanol extract was studied using the micronucleus test.

Seven to nine week-old Swiss mice were used as test animals. Mitomycin C was used to induce chromosomal aberrations, injected intraperitoneally twice at a uniform dosage of 0.003 mg/g body weight, 30 h and 6 h before the mice were sacrificed.

The test extracts, dissolved in 50% dimethylsulfoxide (DMSO), were administered orally at a dosage of 0.2 mg/g mouse, just before injection with mitomycin C. Five mice were used for each test.

Immediately after sacrificing the mice, the bone marrow from the femur was suspended in fetal calf serum. The suspensions were centrifuged at 1000 rpm for 5 min and the cells in the sediment were smeared on glass slides. Three slides were smeared for each mouse. The air-dried slides were stained successively with May-Grunwald and Giemsa stains. The slides were scored under a high-power microscope by counting the number of micronucleated polychromatic erythrocytes (MPCE) per 1000 polychromatic erythrocytes (PCE). The scores obtained with treatment with crude ethanol extract alone were compared with the value on spontaneous mutation (Guevara, 1988) while the scores obtained from treatment with crude ethanol extract and mitomycin C were compared with the control experiment treated only with mitomycin C.

The data on the number of MPCE per 1000 PCE were processed on an IBM PC using Analysis of Variance.

Bioassay for antitumor promoting activity: the Epstein-Barr Virus - Early Antigen (EBV-EA) test (Takasaki, et al. 1995)

The effects of the isolates on EBV-EA formation was assayed using Raji cells (virus non-producer), the EBV genome-carrying human lymphoblastoid cells which were cultivated in 10% FBS RPMI 1640 medium (Nissui). The indicator cells (Raji) (1 x 10^6/ml) were incubated at 37°C for 48 h in 1 ml of the medium containing n-butyric acid (4 mM) as inducer, TPA (32) pmol solution as promoter in 2 μl of DMSO and a known amount of test samples dissolved in 5 μl of DMSO. Smears were made from the cell suspension. The activated cells were stained with high titer EBV-EA positive sera from nasopharyngeal carcinoma patients and detected by an indirect immunofluorescence technique. In each assay, at least 500 cells were counted and the tests were repeated twice. The average induction was compared with that of the positive control experiments with n-butyric acid and TPA, in which EBV-EA induction was ordinarily around 40%. These values were taken as the positive control (100%). The viability of cells were assayed against that of treated cells by the trypan-blue staining method.

RESULTS AND DISCUSSION

Figure 1 summarizes the treatment effects of the crude extract and solvent fractions on carrageenan-induced inflammation.. Indomethacin, a known anti-inflammatory agent, gave an inhibition of 95%. The crude ethanol extract, at a dosage of 1 mg per g body weight, reduced inflammation by 79%. However, such activity was reduced upon solvent fractionation, with the methanol fraction reducing inflammation by 60%, CCl_4 fraction by about 45%, and the hexane fraction by only 23%. These observations may suggest additive and/or some synergistic mechanism among the components in the different solvent fractions.

Figure 2 summarizes the results obtained with the micronucleus test. The relatively high count of MPCE observed with mitomycin C (10.8) is consistent with its mutagenicity. The crude ethanol extract when administered alone gave a count of 1.76 MPCE per 1000 PCE at a dosage of 0.2 mg/g mouse, a count of the same magnitude as that observed for spontaneous mutation, which is 1.78 MPCE/1000 PCE (Guevara, 1988). This observation suggests that the crude extract did not induce chromosomal aberrations and therefore, is non-mutagenic. When the crude extract was administered together with mitomycin C, a 50% reduction in the number of MPCE was observed at a dosage of 0.2 mg/g mouse, suggesting that the extract is antimutagenic.

Figures 3 summarizes the treatment effects of the crude ethanol extract on EBV-EA activation. At a dosage of 100 μg/ml, the crude ethanol extract inhibited EBV-EA activation by 100%. However, all the cells died, indicating the cytotoxicity of the extract at this concentration. Tenfold dilution resulted to a reduction in the EBV-EA activation by 56% with a cell viability of 70%. Further, dilution to 1 μg/ml lowered the observed % inhibition to only 16%. At a very low dosage of 0.1 μg/ml, no activity was observed. These data suggest moderate antitumor-promoting activity of the crude ethanol extract. However, at relatively higher dosage, the extract is cytotoxic.

Figure 4 summarizes the antitumor-promoting activities of the solvent fractions. The observed % inhibitions indicate that the three solvent fractions are very active (100% inhibition) only at high concentration (100 μg/ml). With tenfold dilution, only the CCl_4 fraction exhibited a significant inhibition of 45.3%. These observations suggest weak antitumor-promoting activities of the solvent fractions. However, the high viability of the cells (more than 60%) indicates that the solvent fractions are not cytotoxic unlike the crude ethanol extract. This again may suggest the synergistic and/or additive action of the components in the three solvent fractions.

CONCLUSIONS AND RECOMMENDATIONS

The antimutagenic data obtained in this research indicate the significant anti-inflammatory, and antitumor-promoting activities of the crude ethanol extract of mahogany seeds. The data further show that the solvent fractions exhibited less anti-inflammatory and antitumor-promoting activites, which may suggest synergistic and/or additive action of the bioactive compounds.

One very significant observation was the cytotoxicity of the relatively high concentration of the crude ethanol extract when tested by the Epstein-Barr virus bioassay. Such cytotoxicity was lost upon solvent fractionation. The crude extract, however, was not mutagenic. In fact, it was antimutagenic.

Extraction and fractionation procedures are underway in the laboratory of the authors to isolate the pure bioactive compounds from the extract of mahogany seeds.

ACKNOWLEDGMENT

The extraction/fractionation and the anti-inflammatory and antimutagenic studies were conducted at the Natural Products Research Laboratory, Institute of Chemistry, University of the Philippines Diliman (UPD) as the undergraduate thesis of A. Apilado (B. S. Chem, 1996), with Dr. A. Guevara as adviser, while the antitumor-promoting activity studies were conducted at the Kyoto Prefectural University of Medicine.

A. Guevara acknowledges the University of the Philippines Diliman, the Japan Society for the Promotion of Science, and the Department of Science and Technology for the research visit to the Kyoto Pharmaceutical University and the Kyoto Perfectural University of Medicine.

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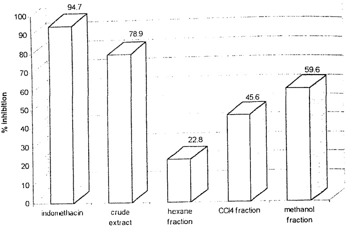

Fig 1. **Treatment Effects of the Crude Extracts of Mahogani on Carrageenan-induced Inflammation.**

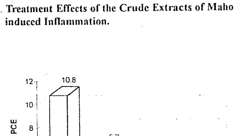

Fig 2. **Antimutagenic Activity of the Crude Extract of Mahogani.**

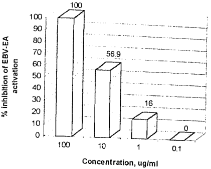

Fig 3. Treatment Effects of the Crude Extracts of Mahogani on EBV-EA Activation.

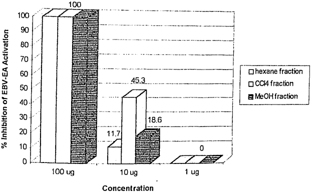

Fig 4. Treatment Effects of Extracts of Mahogani on EBV-EA Activation.

TOXICITY OF FUNGICIDES NIMROD AND DITHANE M-45 ON MITOTIC CELLS OF ALLIUM CEPA L.

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ABSTRACT

The effects of the fungicides Nimrod and Dithane M-45 were studied on root mitosis of Allium cepa L. Actively dividing root tips were treated with 0.05, 0.10, 0.15 and 0.20% of Nimrod and 0.15, 0.20, 0.25 and 0.30% of Dithane M-45 for 1.5, 3.0, 4.5 and 6.0 hours durations. Controls were included in both the experiments. Observations were made in four roots of each treatment combination and the data recorded via Data Entry II for analysis by SPSS/PC⁺ programme. Analysis of variance for each experiment was performed in 4 x 5 factorial randomized complete block design. Both the fungicides progressively inhibited the mitotic activity as the concentrations increased and the period of treatment was prolonged. The frequency of abnormal cells was directly proportional to the concentration and period of treatments.. The chromosomal abnormalities induced by these fungicides were stickiness, fragmentation, bridge formation, lagging of chromosomes, c-mitosis, polyploidy, multipolar anaphase, star metaphase and other abnormalities of cell division. Nimrod induced higher percentage of fragmentation as compared to Dithane M-45. Lagging of chromosomes and bridges were observed only occasionally in both the fungicides.

INTRODUCTION

In many developing countries, the use of agrochemicals for the control of various diseases has been increasing considerably since the last few decades. The value of these pesticides has been shown by greater agricultural productivity in most cases, such as cultivation of fruits and vegetables and their consumption is expanding in urban areas. There are, however, a number of drawbacks associated with pesticide use. Besides causing environmental pollution, major disadvantage of using these pesticides is genetic disbalance in plants. Nimrod is a systemic and wide spectrum fungicide, effectively used against many species of powdery mildews. The active ingredient of Nimrod is bupirimate. Dithane M-45 is the non-systemic fungicide also known as mancozeb. It is effective against any fungal diseases including early leaf spot and leaf rust. It gives better results against initial infection and is applied on peanuts, fruits, vegetables and field crops. In this paper genotoxic effects of two fungicides, viz., Nimrod and Dithane M-45, are reported.

MATERIALS AND METHODS

Medium sized healthy bulbs of a local cultivar of onion, Allium cepa L. were selected and their basal portions shaved, placed on the beakers filled with water, kept at room temperature to obtain new and actively growing roots. After 3 or 4 days, actively growing roots were treated with the fungicides in several concentrations for varying durations. The concentrations of Nimrod were: control, 0.05, 0.10, 0.15 and 0.20% and of Dithane M-45: control, 0.15, 0.20, 0.25 and 0.30%. Each concentration was applied for 1.5, 3.0, 4.5 and 6.0 hours durations, and each treatment combination was replicated thrice. After the treatment, root tips were transferred to 2% aceto-orcein and stored at 4 °C for 24 hours. Hydrolysis was done in 1N HCl for 3-4 minutes at 60 °C. Slides were prepared by using squash technique without heating. From each of the four root tips, 2-3 slides were prepared and observations were made in ten random fields per slide and the data were recorded via Data Entry II for analysis in a personal computer. The percentage of abnormal cells were calculated out of dividing cells. Frequencies of total cells, dividing cells and various kinds of abnormal cells were noted and saved in a DE II (data entry II) programme for transformation and statistical analysis by SPSS/PC$^+$. The abnormalities recorded in percentages of dividing cells were transformed by arcsine method before further analysis.

RESULTS AND DISCUSSION

The effects of different treatment combinations of Nimrod and Dithane M-45 on the mitotic division in the root meristems of Allium cepa are given in Tables 1 and 2, respectively. A decrease in mitotic index was observed with the increase in concentrations of both the fungicides. Also the proportion of several chromosomal abnormalities increased with the increase in concentration of fungicides. The frequency of almost all chromosomal abnormalities was directly related to the duration of treatment as well.

Polyploidy, c-mitosis and disturbed metaphase were the most common abnormalities induced by these fungicides. The higher percentage of polyploidy (Figure 1a and 1b) and disturbed metaphase (Figure 1c) was observed in 0.20% of Nimrod and 0.3% of Dithane M-45. Disturbed Metaphase is characterized by the scattering of chromosomes all over the cell and by the appearance of star shaped metaphase, which is believed to be due to complete disturbance of spindle fibers (Amer and Ali, 1986). In the present study, this abnormality was very rare. The induction of polyploidy increased with the increase in the concentration and duration of treatment. C-mitosis (Figure 1d) is due to disturbance in spindle activity, causing a deviation from the normal bipolar mitosis. It is also produced after treatment with other pesticides and chemical compounds like Gesagard and Igran (Topaktas and Rencuzogullar, 1991).

Stickiness (Figure 1e & 1f) is reported to be a physiological effect on the chromosomes during division (Savage, 1975) and has been considered as a category of chromatid aberrations (Klasterska, et al., 1976). Chromosome stickiness was observed in different treatment combinations of both the fungicides. Nimrod induced stickiness in all the durations of treatment (Table 1) while in Dithane M-45 stickiness was not observed in 1.5 hours treatment (Table 2). The frequency of stickiness was found to be dose and time dependent. Stickiness was also observed by the treatment with other pesticides such as Glean (Badr and Ibrahim, 1987), Garlon-4 (El-Khodary, et. al., 1989) and S Triazine herbicide Turbutryn (Badr, 1986). Both fungicides caused fragmentation of chromosomes in different concentrations and for different durations of treatment. Nimrod induced fragmentation more frequently than Dithane M-45. Nimrod, in all concentrations induced fragmentation whereas it was observed only in higher concentrations i.e., 0.25 and 0.30% of Dithane M-45. It was also observed that after treating Allium cepa roots with pesticide Igran (El-Khodary, et. al., 1987), Endrin and Benlate (Sattar and Vahidy, 1988). Fragmentation of chromomosomes, observed in the present study was due to the direct attack of Nimrod and Dithane M-45 on the hereditary material i.e., DNA of the chromosomes, which resulted in the breaking of chromatids/chromosomes.

Lagging of chromosomes and bridges were also observed in low frequencies. Bridges (Figure 1) may be formed due to chromosome breakage and reunion (Tomkins and Grant, 1972). Bridge formation was also observed in wheat mitosis after treatment with fungicides Vitavax-200 and Dithane S-60 (Najjar and Soliman, 1980). Abraham and Koshi (1979) suggested that stickiness of chromosome resulted in anaphase bridges and subsequent failure of free anaphase separation. Nimrod induced bridges in 0.15 and 0.20 concentration in 3-hour treatment (Table 1) and Dithane M-45 in 0.25% concentration in 6-hour treatment (Table 2). Laggards (Figure 1) were observed in 0.10, 0.15 and 0.20% of Nimrod and 0.20, 0.25 and 0.30% of Dithane M-45 treatment. Studies on the effects of Methylparathion and Trimiltox on mitosis of Allium cepa don by Ahmed and Yasmin (1992) reported the high frequencies of laggards. Lagging of chromosomes is due to failure of chromosomes to reach at metaphase plate and due to improper formation of spindle fibers in the anaphase stage. Abnormalities like stickiness, chromosomal fragmentation, anaphase bridges and lagging chromosomes are also produced by the effect of magnetic field on the living cells of Allium cepa (Runthala and Bhattacharya, 1991).

Additional abnormalities of cell division, including multipolar division, adnormal arrangement of chromatic threads, disturbed anaphase etc., were also observed. Abnormal prophase was characterize by abnormal arrangement of chromatic threads. Multipolar division (Figure 1) due to partial suppression of spindle action was also observed in Alium cepa root tip cells after treatment with IPC.

Analysis of variance for both the fungicides revealed that there was a significant difference among different concentrations and different durations of the treatments with respect to mitotic index and percentage of abnormal cells (Table 3). Since two independent experiments were planned for Nimrod and Dithane M-45, each as 4x3 factorial in Randomized Complete Block Design (RCBD), analysis of variance (ANOVA) was conducted to check the differences in mitotic index and abnormalities of dividing cells, among various levels of concentrations (C), durations (D) and their interaction C x D. The results of ANOVA for mitotic index (Table 3) revealed highly significant differences among the levels of both the variables. However, the results of interaction were different. For Nimrod the interaction (C x D) was highly significant ($p < .000$), suggesting the inconsistent, i.e. non additive behavior of levels of C with that of D, while for Dithane M-45 the interaction was non significant ($p = 0.1$) indicating that the trend in several concentrations was consistent in varying durations. On the other hand, ANOVA for Arcsine transformed percentage of abnormal cells out of total dividing cells revealed highly significant differences among the levels of two factors and their interaction for both the fungicides studied.

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Table 1. Arcsine Transformed Percentage of Various Chromosomal Abnormalities in Mitotically Dividing Cells of Allium Cepa after Exposure to Different Treatment Combinations of Nimrod.

Treatment (hour)	Conc. (% age)	Mitotic Index	Stickiness	Fragmentation	Bridges	Laggards	Polyploidy	Other Abnormalities During		
								Prophase	Metaphase	Anatelophase
1.5	Control	14.44	0	0	0	0	0	0	0	0
	0.05	14.49	5.48	3.69	0	0	2.73	3.11	9.54	0
	0.10	14.11	9.59	0	0	3.11	3.03	6.48	16.63	0
	0.15	13.34	12.94	0	0	7.13	3.29	8.26	13.18	3.11
	0.20	12.94	7.90	6.53	0	3.29	12.91	11.70	15.65	6.87
3.0	Control	15.08	0	0	0	0	0	0	0	0
	0.05	14.31	9.60	2.18	0	0	9.97	9.97	6.24	5.33
	0.10	13.54	7.66	3.19	0	0	11.58	7.03	11.54	10.23
	0.15	12.94	13.40	11.77	2.43	0	13.40	13.23	14.19	3.44
	0.20	12.40	12.87	14.50	2.58	0	15.34	10.69	19.02	4.50
4.5	Control	14.61	0	0	0	0	0	0	3.34	0
	0.05	13.93	8.56	3.07	0	0	7.66	12.03	10.74	6.63
	0.10	12.88	10.29	11.72	0	0	12.89	3.96	13.80	6.89
	0.15	12.27	12.00	12.17	0	0	15.14	12.00	15.14	12.00
	0.20	11.75	14.28	11.31	0	3.03	18.43	13.79	16.59	15.27
6.0	Control	15.21	0	0	0	0	0	0	0	0
	0.05	13.83	8.95	6.10	0	0	7.29	7.84	12.45	8.95
	0.10	13.16	12.91	10.24	0	0	18.80	12.91	13.80	11.52
	0.15	11.99	14.28	11.39	0	0	16.42	14.28	16.42	14.28
	0.20	11.40	17.10	14.27	0	0	19.66	15.94	18.41	12.86

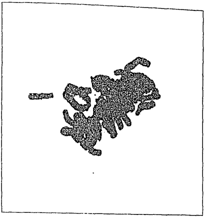
Table 2. Arcsine Transformed Percentage of Various Chromosomal Abnormalities in Mitotically Dividing Cells of Allium Cepa after Exposure to Different Treatment Combinations of Dithane M-45.

Treatment (hour)	Conc. (% age)	Mitotic Index	Stickiness	Fragmentation	Bridges	Laggards	Polyploidy	Other Abnormalities During		
								Prophase	Metaphase	Anatelophase
1.5	Control	16.64	0	0	0	0	2.76	0	0	0
	0.15	16.51	0	0	0	0	5.76	0	8.42	5.39
	0.20	16.29	0	0	0	0	9.48	2.79	5.71	8.37
	0.25	15.79	0	0	0	0	11.42	4.20	10.18	10.18
	0.30	15.24	0	0	0	0	13.03	9.36	12.93	11.76
3.0	Control	16.77	0	0	0	0	0	0	0	0
	0.15	16.33	5.48	0	0	0	2.41	3.15	8.63	3.15
	0.20	15.90	8.65	0	0	3.15	9.86	2.88	6.58	2.60
	0.25	15.36	3.92	0	0	3.11	12.78	6.30	11.38	6.30
	0.30	15.10	11.53	3.29	0	0	12.92	6.34	10.96	10.14
4.5	Control	16.69	0	0	0	0	0	0	2.47	0
	0.15	16.03	0	0	0	0	9.64	5.03	8.27	6.79
	0.20	15.52	2.76	0	0	0	11.98	8.56	9.72	9.72
	0.25	14.98	10.24	3.03	0	0	14.72	9.07	11.51	7.21
	0.30	14.37	13.77	6.14	0	2.99	13.77	9.03	12.83	10.29
6.0	Control	16.56	0	0	0	0	0	0	0	0
	0.15	15.88	5.58	0	0	0	10.75	3.92	10.75	2.76
	0.20	15.17	9.87	0	0	0	14.03	11.24	12.67	9.87
	0.25	14.53	12.08	10.73	3.19	0	18.37	9.49	6.29	12.14
	0.30	13.67	12.84	10.55	0	2.79	20.68	6.08	15.31	14.40

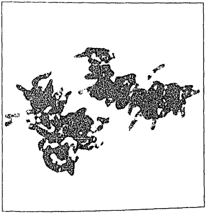
Table 3. Analyses of Variance for Mitotic Index and Abnormal Mitosis in Root Tips of *Allium Cepa* after Treatment with Fungicides, Nimrod and Dithane M-45, in Varying Concentrations and Durations.

Sources of Variation	Degrees of Freedom	Mean Squares & (Significance of F)			
		Mitotic Index		Abnormal Cells	
NIMROD					
Replication	2	5.73		7.54	
Concentrations (C)	4	14.47	(0.000)	2453.51	(0.000)
Durations (D)	3	2.27	(0.000)	265.94	(0.000)
C x D	12	0.46	(0.000)	18.51	(0.000)
Error	38	0.06		2.88	
DITHANE M-45					
Replication	2	3.11		6.08	
Concentrations (C)	4	8.03	(0.000)	1392.82	(0.000)
Durations (D)	3	2.53	(0.000)	196.13	(0.000)
C x D	12	0.21	(0.100)	23.84	(0.000)
Error	38	0.008		5.75	

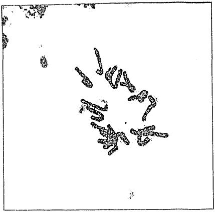
Figure 1. Different Abnormalities Produced by Nimrod and Dithane M-45 in Mitotic Cells of *Allium Cepa* L.:



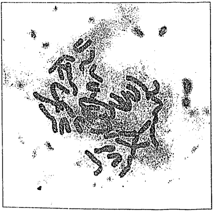
a Polyploidy at Metaphase.



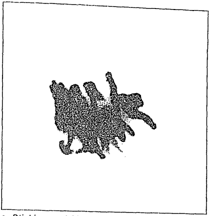
b. Polyploidy at Anaphase.



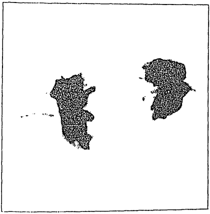
c. Disturbed Metaphase.



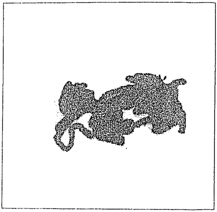
d. C-mitosis.



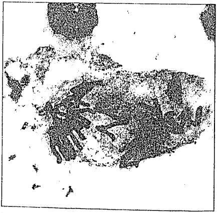
e. Stickiness at Metaphase.



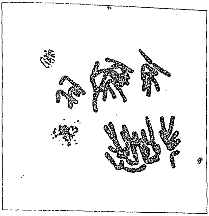
f. Stickiness at Anaphase.



g Anaphase Bridges.



h. Laggards at Anaphase.



I Multipolar Division.

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PHILIPPINE JOURNAL OF SCIENCE

IRON ABSORPTION FROM FVRICE-IRON FORTIFIED

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ABSTRACT

Iron bioavailability from unfortified (Meal A) and FVRice-iron fortified (Meal B) based meals was determined by simultaneous determination of ^{55}Fe and ^{59}Fe in blood in 20 healthy human subjects. FVRice-iron fortified was prepared by mixing 1 part of premix rice to 200 parts by weight of ordinary rice. The total iron content of the FVRice-iron fortified alone (uncooked) was 5.71 mg/100g; 2.84 mg/100g, when given as a mixed diet. The geometric mean iron absorption of 6.88% from Meal A was significantly higher than that obtained for Meal B of 5.41%. However, the amount of iron absorbed increased significantly from 0.30 to 0.40 mg ($<.01$) when the total non-heme iron content was considered. Consumption of 120-150 g rice daily might satisfy about 65-81% of the iron requirements of 1-3 yr children, thus meeting more than the goal of 40-50% RDA for this age group. The use of ferrous sulfate as fortification iron to rice seems to be effective, supplying an appropriate amount of absorbable iron.

INTRODUCTION

Anemia continues to be public health problem in the Philippines. Latest surveys conducted by the Food and Nutrition Research Institute (FNRI) showed a decrease in overall prevalence of anemia from 37.2% in 1987 to 28.9% in 1993 but remained high in infants (49.2%), the elderly (45.6%), pregnant (43.6%) and lactating women (43%) (Kuizon et al, 1994). This may be due not only to inadequate intake of dietary iron but also to its poor bioavailability. Fortification of food products with iron is currently used, or planned to be introduced, in many countries not primarily to treat but to prevent Fe deficiency (INACG, 1977). In the Philippines, rice, being the dietary staple of the majority of the population, is a suitable vehicle for fortification. Recently, the Nutritional Product Development Section, FNRI, Department of Science and Technology modified the technology for iron fortified rice developed by Payumo et al. (1982) on a laboratory scale. The enriched rice, known as FVRice-iron fortified, provides 3 mg iron per 100 g. This would meet almost 100% of the RDA for iron for a male adult consuming 300 g rice and about 40-50% of the RDA for 1-3 year old child consuming 120-150 g rice. It is necessary, however, to study the bioavailability of iron from the FVRice-based meal before the fortified rice is introduced as an intervention program to improve the iron status of the population.

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This study aims to measure iron absorption from FVRice-iron fortified based meal using the radioisotope extrinsic tag method to determine to what extent, if any, absorption was increased by the proposed fortification.

MATERIALS AND METHODS

Subjects

Ten male and 10 female volunteers, aged 21-37 years, were used as subjects. All were in good health and had no history of disorders known to influence the gastrointestinal absorption of iron. Only females with negative results for pregnancy test were included in the study. None of the subjects were anemic but iron status as assessed by serum ferritin measurements varied widely. A written informed consent was obtained from the subject before the study after receiving full explanation of the nature and purpose of the study. The study was approved by the National Health Research Ethics Committee, Philippine Council for Health Research and Development, DOST.

Preparation of Enriched Rice and Test Meals

Rice was purchased from the local market. The grains were made to pass through a No.11 sieve to remove as much broken grains as was possible. Anhydrous ferrous sulfate obtained from a local distributor was used as fortification iron.

Premix rice was prepared by coating the rice uniformly with 37 kBq 59Fe-labeled ferrous sulfate using a compound for ethyl cellulose-methyl cellulose-hexane-isopropyl alcohol as coating solution. The coated rice were air-dried turning the grains once in a while to prevent lump formation.

FVRice-iron fortified was prepared by mixing 1 part premix rice to 200 parts by weight of ordinary rice. The total iron contents of the ordinary rice, premix rice and FVRice-iron fortified are shown in Table 1.

The meals were composed of rice, fried "tulingan" (Auxis thazard), "ginataang lanka" (jackfruit boiled with coconut milk) and ripe mango prepared to supply 1/3 of the RDA for energy, protein and iron following the usual proportions found in food consumption surveys. Nutrient composition in terms of energy, fat, protein, iron, and ascorbic acid was initially estimated using the Philippines Food Composition Table Handbook (FCT, 1990). All the food components were weighed and cooked in aluminum pots individually per subject to avoid distribution problem with the radioactivity. The mean non-heme iron content of the unfortified rice based meal (Meal A) was 4.0 mg while that of the FVRice-iron fortified based meal (Meal B) was 7.4 mg. Meal A was labelled with 2 uCi $^{55}FeCl_3$ and Meal B with 1uCi $^{59}FeCl_3$.

The reference dose of ferrous ascorbate containing 3 mg Fe and 30 mg ascorbic acid in 10 ml of .01 M CHI was labelled with 1 uCi $^{59}FeCl_3$.

The meals were also analyzed for total iron (AOAC, 1965), ascorbic acid (AOAC, 1965), phytic acid (Lowry and Lopez, 1946), and tannic acid (Sharman et al, 1953). Table 2 shows the nutrient composition of the test meals.

Feeding Regimen

The protocol for feeding, administration of radioisotope, and collection of specimens is schematically presented in Figure 1. All meals were eaten between 0700 and 0900 AM the laboratory after a 10 h overnight fast for 2 consecutive days as follows: Day 1: Meal A (^{55}Fe), and Day 2: Meal B (^{59}Fe). No food drink was allowed for at least 3 h after the intake of the labelled meal. After 14 days blood was extracted from the subjects to determine their hematological profile and to measure circulating red cell radioactivity. Then on two consecutive mornings (Day 15 & 16), reference doses of ferrous ascorbate containing 3 mg Fe and 30 mg ascorbic acid labelled with ^{59}Fe were given to the subjects to measure their absorptive capacity. A final blood sample was drawn 2 weeks later (day 29) to measure the increased radioactivity in circulating red cells. Hemoglobin concentration was determined by the cyanmethemoglobin method (ICHS, 1978) and serum ferritin was measured by enzyme-linked immunoassay (ELISA) method (Flowers et al, 1986).

Iron Absorption Measurements

The relative absorption of ^{59}Fe was calculated from the radioactivities in the food consumed and in blood samples drawn two weeks after serving the labelled meals. Duplicate 5 mL samples of whole blood and duplicate portions of suitable standard iron solutions were prepared for differential counting of ^{55}Fe and ^{59}Fe by using a modification of the method of Eakins and Brown (1966). The radioactivity of the digested blood samples were read in the Liquid Scintillation Spectrometer (Packard, Tri-carb Model 3255) available at the Philippine Nuclear Research Institute, DOST. Percentage iron absorption was calculated blood volume estimated from sex, height, and weight of the subjects on the assumption that 80% of the absorbed radioactivity was incorporated into the circulating red cells (Nadler et al, 1962, Botwell et al, 1979).

Data Analysis

Because the distribution of iron absorption data is positively skewed when expressed as a percentage of the administered dose, statistical analysis was performed on logarithms and the result reconverted to antilogarithms to recover the original units. Student's test was used to compare iron absorption between unfortified rice and FVRice-iron fortified-based meals. Simple correlation analysis was used to test the relationship between iron absorption from the reference dose and serum ferritin levels.

RESULTS AND DISCUSSION

The subjects were served two identical rice-based 'lunch' meals on two consecutive mornings. Meal A was not fortified and contained 3.91 mg non-heme iron for males and 4.1 mg non-heme iron for females. The other meal (Meal B), containing RVRice-iron fortified, had a non-heme iron content of 9.04 and 5.70 mg for males and females, respectively. The hematological characteristics of the subjects and the results of iron absorption are shown in Table 3. The serum ferritin level averaged 100.7 ug/L (range 17.5 - 264.9 ug/L) and mean absorption from reference dose (R) was 20.2%. The mean iron absorption from Meal A was 1.11% for males and 4.82% for females while 0.77 and

4.32% absorption were obtained from Meal B for males and females, respectively. Significant differences on iron absorption between males and females were observed both from Meal A (t=7.560, p>.05) and Meal B (t=13.186, p<.01). The difference may probably be attributed to the greater need of women for iron to replace losses especially during menstrual period. As shown in their serum ferritin levels, males had more adequate iron stores (150 ug/L) than females (67.4 ug/L. The over-all mean iron absorption from the unfortified rice-based meal of 2.31% (range 0.5-16.5%) was significantly higher than that obtained from FVRice-iron fortified based meal of 1.82% (range 0.2-14.10%). These results are consistent with published data on the effect on absorption of increasing the iron content of meals (Botwell et al, 1979, Layrisse et al, 1973).

The variability in iron absorption among individuals refllected differences in iron status as evidenced by the inverse relationship between iron absorption from the reference dose and serum ferritin levels (r=0.61, p < .01). To decrease the influence of individual differences, absorption values were related to the reference dose (A/R or B/R) and the mean absorption ratios obtained were 0.17 and 0.13 from Meal A and Meal B, respectively.

Estimates of the amount of iron absorbed from each meal in iron-deficient children was obtained by correcting the percentage absorption to a reference value of 60% (Stekel, 1984). Table 4 shows the estimated iron absorption from the unfortified and FVRice-iron fortified based meals adjusted for iron status. The geometric mean values obtained are 6.88% for Meal A and 5.41% for Meal B. When the non-heme iron contents of 4 mg in Meal A and 7.4 mg in Meal B were considered, however, the amount of absorbed iron were 0.30 mg iron from Meal A and 0.40 mg iron from Meal B. The 0.10 mg increase (33%) in absorbed iron was significant (t=3.12, p<.01). The daily requirement for absorbed iron has been estimated to be 0.73 mg for 1-3 year children (RDA, 1989). Therefore, consumption of 120-150 g of FVRice daily might satisfy about 65-81% of the iron requirements of 1-3 year old children. This result is higher than the goal of meeting 40-50% as stated earlier.

Rice contains inhibitory ligands (phytates) that bind iron and reduce its absorption. Ferrous sulfate appears to enter the common non-heme iron pool in the meal and is therefore affected by the enhancing and inhibitory ligends in the same way as all the iron in the pool. Therefore actual availability for rice or any cereal depends not on the iron compound added but more importantly on the composition of the diet and on the interaction between these ligands and the common pool (Hurrell et al, 1989).

CONCLUSION AND RECOMMENDATION

The study showed that although the percentage of non-heme iron absorption from FVRice-iron fortified based meal decreased, the absolute amount of non-heme iron absorbed increased because of the higher iron content. The 33% increase in the amount of absorbed iron was observed by fortification. Results obtained in this study shows ferrous sulfate maybe a good iron fortificant. The use of other iron compounds like Na-Fe ethylenediamenetetraacetic acid (EDTA) or iron diglycinate which have been found to be relatively independent of meal composition may also be considered for iron fortification in humans. Other vehicles, i.e. sugar, fish sauce, etc, may also be used for iron fortification. Moreover, inclusion of iron enhancers (ascorbic acid) into and reduction of inhibitors (phytates, tannins) from the rice-based meal may be done to improve absorption.

ACKNOWLEGEMENT

This work was supported by a research grant from the Philippine Council for Health Research and Development, DOST.

The authors wish to thank the members of the technical committee of the FNRI for editing the manuscript, Dr. Rebecca Monte for medical examination of subjects, Misses Juanita R. Madriaga and Josefina D. Desnacido for their assistance in serum ferritin determination, Ms. Revelita L. Cheong for statistical analysis of data and Mr. Teofilo Garcia of FNRI, Zoilo B. Villanueva and Adorie D. Sabenecio of FNRI for their technical assistance.

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Table 1. Analyses of ordinary rice, premix and enriched rice for moisture and iron contents.

Sample	Moisture g/100g	Iron mg/100g
Ordinary rice	11.1	0.62
Premix	12.7	1,053.0
Enriched rice	13.0	5.71

Table 2. Nutrient composition of FVRice iron-fortified based meal.

Nutrient	Per 100 g
Protein (g)	7.15
Energy (kcal)	185.8
Fat (g)	3.40
Total Iron (mg)	2.84
Non-heme iron (mg)	2.42
Ascorbic acid (mg)	16.0
Phytic Acid (mg)	138.0
Tannic Acid (mg)	146.0

Table 3. Iron absorption from FVRice-iron fortified.

Subject	Sex/Age	Hb g/dL	Packed-cell volume %	Serum Firritin ug/dL	Iron Absorption % of dose			Absorption Ratio
					Reference Dose	Meal A	Meal B	B:A
1	M21	16.0	46	17.5	37.3	11.2	5.2	0.46
2	M22	16.9	46	211.3	12.1	0.6	0.2	0.33
3	M23	15.7	41	264.9	12.2	1.5	1.2	0.80
4	M35	12.8	38	124.5	10.6	0.3	0.5	1.67
5	M35	17.1	48	117.9	4.2	0.3	0.5	1.67
6	M35	13.9	43	216.0	16.4	0.8	0.2	0.25
7	M33	16.4	46	206.7	10.0	0.6	0.5	0.83
8	M34	15.8	48	244.9	17.6	0.5	0.5	1.00
9	M26	15.2	44	158.0	14.1	1.3	0.6	0.46
10	M24	14.6	40	239.0	27.4	9.9	7.5	0.76
Mean* ±SD	29	15.4	44	150.4 ±2.2	13.92 ± 1.81	1.11 ±3.66	0.77 3.40	0.82 ±0.51
11	F24	13.7	41	68.8	32.8	9.8	6.7	0.68
12	F34	13.8	40	94.8	16.2	3.0	4.1	1.37
13	F35	14.0	37	31.4	36.4	3.1	2.1	0.68
14	F29	13.9	38	104.0	20.2	0.5	0.8	1.60
15	F27	14.5	39	94.4	36.5	14.0	11.0	0.79
16	F27	15.2	42	74.2	21.6	4.1	3.3	0.80
17	F37	14.0	39	46.3	39.8	7.1	6.8	0.96
18	F35	12.7	38	80.2	31.2	1.9	2.0	1.05
19	F27	13.3	41	72.8	36.8	16.5	14.1	0.85
20	F27	14.6	40	48.8	31.9	11.5	7.1	0.62
Mean* ±SD	30	14.0	40	67.4 ±1.46	29.19 ±1.36	4.82 ±2.95	4.32 ±2.41	0.94 ±0.32
Over-all MEAN ±SD	29	14.7	42	100.74 ±2.09	20.16 ±1.82	2.31 ±4.00	1.82 ±3.91	0.88 ±0.42

[1] Meal A, unfortified; Meal B, FVRice-iron fortified.

Table 4. Mean iron absorption from unfortified (A) and FVRice iron-fortified (B) based meals.

Test meal	Non-heme iron content mg	Mean Iron Absorption % of Dose	Absorption	
			% A_{60}*	mg Fe
A	4.0	2.31 ± 4.00	6.88 ± 2.60	0.30
B	7.4	1.82 ± 3.91	5.41 ± 2.72	0.40

*Corrected to 60% REF absorption

DAY							
1	2	3 - 13	14	15	16	17 - 28	29
Meal A 74kBq	Meal B 37kBq	No Feeding	Blood Extraction	RD B 37kBq	RD B	No Feeding	Blood Extraction
55_{FE}	59_{FE}			59_{FE}			

Meal A - Unfortified rice-based meal
Meal B - FVRice-iron fortified-based meal
RD Reference Dose

Figure 1. Feeding protocol for each subject

ANNUAL RECURRENCE OF FUSARIA IN THE RHIZOSPHERE OF ORNAMENTAL CROPS GROWN IN DIFFERENT SOILS OF ALLAHABAD

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ABSTRACT

Annual recurrence of fusaria in the rhizosphere of ornamental crops grown in three different regions of Allahabad (Medical College, Chandra Shekhar Azad Park and Botany Department) was studied during 1993 (January-December). Variation in the number of Fusarium colonies was observed throughout the year. The study revealed that the atmospheric temperature, soil pH and the host species play an important role in the growth and development of fusaria. Atmospheric temperature ranging from 27-36 °C, pH 7.0-7.8 and the host Chrysanthemum favored most the growth of fusaria.

INTRODUCTION

Environment plays an important role in the annual recurrence of soil-borne pathogens. Adverse environmental conditions can limit the annual recurrence of pathogen, reduce the density and potency of the inoculum, thus, indirectly affecting the chances of disease development. Mustafee and Chattopadhay (1985) reported that *Fusarium oxysporum* f.sp. conglutians (cabbage wilt pathogen) survived best in soil at pH 5-6, temperature 20-24 °C and moisture 40% for 16 months with host tissue and 18-19 months when cabbage tissue had been added. The study of relationship of pathogens with environment can be of considerable help in designing techniques to solve the problem of soil-borne diseases. The effect of various environmental factors on the distribution of pathogenic fungi in soil has been studied by several workers (Games and Domesh, 1969; Singh and Bhargava, 1981; Khati et al, 1982). The soil pathogens encounter two kinds of environment viz., the atmosphere and the sub-surface soil environment. The edaphic environment has several components viz., moisture, pH and nutrients while the atmospheric environment includes humidity, temperature and rainfall.

Singh and Chaube (1975) found that at temperature of 30°C and 35°C, there was more loss of spores as compared to hyphal propagule of *Fusarium nudum*. El-Abyad and Saleh (1971) observed that the temperature ranging from 25-30°C favored the germination of spores, germ tube elongation, sporulation and growth of *F. Oxysporum* f. *sp. vasinfectum*. They also found that the activity of *F. oxysporum* f. sp. *vasinfectum* was maximum in soil with neutral pH or with medium texture. Kraft and Robert (1969) reported that the severity of root and foot rot of pea caused by *F. solani* f. *sp. pisi* was suppressed in soils with high moisture content. Mukhopadhyay and Gupta (1991) also observed that competitive saprophytic activity of Fusarium udum in soil declined rapidly with increase in soil moisture. In the present investigation, an attempt has been made to find out the annual recurrence of fusaria in the rhizosphere soils of ornamental plants grown in different regions of Allahabad.

MATERIALS AND METHODS

In order to study the annual recurrence of fusaria, random soil samples were collected regularly every month for a year (January-December, 1993) from the garden of Botany Department, University of Allahabad, Chandra Shekhar Azad park and Moti La] Nehru Medical College, Allahabad. Soils from 7.5, 15.0, 22.5 and 30.0 cm depth were scraped from the four sides of the pits using a sterilized sharp steel blade. The samples collected in pre-sterilized polythene bags from various depths were then mixed together thoroughly to make a uniform sample. The Soil was air dried, pulverized and passed through a 2 mm sieve. Isolations were made on modified Czapek's Dox Agar medium (Singh and Nene, 1965) by soil dilution and plate count method.

Meteorological data was obtained from the airforce meteorological station, Bamrauli, Allahabad. The percentage of nitrogen and organic carbon was estimated by Kjeldahl's titration method.

RESULTS AND DISCUSSION

Table 1 shows the texture, pH, moisture content, nitrogen content and organic carbon content of soil. The results show a gradual decrease in the number of *Fusarium* colonies during February to June, minimum being in June. In the present investigation, atmospheric temperature was found to markedly affect the population of *Fusarium* species in the soils. As the temperature increased from March to June, there was a gradual decrease in number of Fusarium colonies, lowest being in the month of June. The population of fusaria in all the three different soil samples increased rapidly from August to October but showed a decreasing trend in November to January. This probably shows that the population of fusaria in all the three soil fell because of excessive heat or cold, indicating that its development in nature was affected by extremes of atmospheric temperature, moisture and humidity. Kannaiyan and Prasad (1975) reported that low moisture levels and submerged conditions reduced the saprophytic colonization and survival of *Fusarium oxysporum* f. *sp. melonis* in soil. The colonization of the pathogen was retarded at 40, 45 and 50 °C while at lower temperatures viz., 25, 30, 35 °C it was prolonged.

It was also observed that the population of *fusaria* in the soil was influenced by the crop species. Aster, Kochia, Balsam and Chrysanthemum were found to favor the growth of *Fusarium*. It has been observed that some species of *fusaria* take advantage of selective environment and susceptible host tissues and increase their incoculum potential substantially as primary colonizer (Short and Lacy, 1974). Natrajan (1978) frequently isolated several species of fusaria from the rhizosphere and rhizoplane of crop plants and concluded that its population was influenced by the age of plants, the soil type and the plant species or variety. Tandon and Tiwari (1982) made a comparative study of soil mycoflora under different crops and reported that some species of fungi were crop specific, probably due to difference in the quality and quantity of root exudates. In the present study, it was observed that the higher number of colonies were present at pH 7.0, which indicates that a neutral soil favors the growth of the this fungus. Kannaiyan and Prasad (1975) observed that the saprophytic activity of *Fusarium oxysporum* f. *sp. melonis* was best at soil reaction ranging between 4 and 8. Oritsejafor (1986) reported that pH ranging from 5 to 7 is most favorable for growth and survival of *Fusarium oxysporum* f. *sp. elacidis* in soil. The effect of nitrogen content and organic carbon on the growth of fusaria could not be ascertained because of negligible variation in nitrogen and organic carbon content in the three different soils. The study, therefore, revealed that the atmospheric temperature, soil pH and the host species play an important role in the growth and development of fusaria. Atmospheric temperature ranging from 27-36 °C, pH ranging from 7.0-7.8 and the host-Chrysanthemum favored most the growth of fusaria.

ACKNOWLEDGEMENT

Senior author is thankful to the Council of Scientific and Industrial Research (CSIR), New Delhi for providing financial assistance.

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Table 1. Effect of climatic and edaphic factors on the annual recurrence of fusaria in the soils of Allahabad during 1993

Month	Plant	Soils pH	Moisture (%)	Nitrogen (%)	Organic Carbon (%)	Atmospheric Temp. in °C		Relative Humidity (%)		Total Rainfall in mm.	Fusarium colonies /g soil
						Min.	Max.	Min.	Max.		
January	1. Salvia	7.7	10.0	1.55	0.49	5.2	26.4	43.0	99.0	41.9	33
	2. Rose	7.2	9.0	1.39	0.33						35
	3. Aster	7.5	7.0	1.40	0.37						36
February	1. Salvia	7.5	10.0	1.39	0.58	4.3	27.0	37.7	99.0	33.1	50
	2. Rose	7.6	18.0	1.39	0.58						51
	3. Aster	7.8	7.0	1.42	0.39						55
March	1. Salvia	7.6	8.0	1.32	0.40	9.4	41.0	23.7	41.3	NIL	39
	2. Rose	7.3	12.0	1.30	0.43						40
	3. Aster	7.7	10.0	1.32	0.51						42
April	1. Salvia	7.1	12.0	1.19	0.61	17.0	44.9	12.3	58.0	11.0	20
	2. Rose	7.0	10.0	1.29	0.52						23
	3. Aster	7.0	6.0	1.20	0.43						24
May	1. Fallow	7.4	2.0	1.09	0.43	22.6	47.3	8.0	53.0	Trace	17
	2. Rose	7.2	2.0	1.11	0.43						14
	3. Kochia	7.4	4.0	1.06	0.60						20
June	1. Fallow	7.2	14.0	1.05	0.48	24.8	44.6	2.3	95.7	111.5	10
	2. Rose	7.1	8.0	1.12	0.27						8
	3. Kochia	7.5	12.0	1.02	0.57						11
July	1. Balsam	7.6	18.0	1.02	0.51	24.0	37.8	57.0	94.0	130.7	12
	2. Rose	7.2	20.0	1.08	0.28						10
	3. Balsam	7.7	10.0	0.93	0.59						13
August	1. Balsam	7.3	13.0	1.23	1.10	23.5	36.3	56.0	64.3	181.3	19
	2. Rose	7.4	7.0	1.26	0.90						15
	3. Balsam	7.5	8.0	1.23	1.00						19
September	1. Balsam	7.2	19.0	1.19	1.12	19.4	35.4	51.0	96.3	228.0	40
	2. Rose	7.2	8.6	1.23	1.20						44
	3. Balsam	7.1	9.6	1.24	1.40						43
October	1. Fallow	7.0	17.0	1.13	1.00	13.0	36.0	31.0	95.0	34.4	61
	2. Rose	7.1	10.0	1.19	0.89						56
	3. Chrysanthemum	7.0	8.4	1.17	0.90						59
November	1. Fallow	7.0	5.0	1.41	0.43	8.8	32.0	33.0	63.0	Trace	37
	2. Rose	6.9	6.0	1.51	0.40						35
	3. Chrysanthemum	6.8	3.0	1.44	0.44						40
December	1. Fallow	6.8	20.0	1.41	0.45	5.0	30.3	29.0	70.7	4.4	38
	2. Rose	6.6	10.6	1.50	0.39						40
	3. Chrysanthemem	6.5	16.0	1.46	0.45						37

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TOTAL MERCURY OF SELECTED FISH SPECIES FROM LAGUNA DE BAY[1]

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ABSTRACT

Dalag Ophicephalus striatus Bloch, kanduli Arius thalassinus Ruppell, bia Amblygobius phalaena Cuvier et Valenciennes and tilapia Tilapia nilotica Linnnaeus collected from Laguna de Bay between Taguig and Binangonan area in August 1989 to July 1990 were analyzed for total mercury by atomic absorption spectrometry.

The highest metal concentration in soft muscle tissue was observed in Dalag followed by kanduli, less in bia and least in tilapia with mean values of 0.021, 0.020, 0.013, and 0.008 ug/g, respectively.

Analysis using two-way ANOVA showed a significant difference in the mean total mercury in ug/g in the difference fish samples, among the different months and the interaction between these two variables. Mean total mercury of the four fish samples were significantly higher in April than in October.

The results show that the levels of total mercury in the fish samples are below the World Health Organization maximum tolerable consumption of mercury in food of 300 ug or 0.3 mg of total mercury per week.

INTRODUCTION

Mercury is a unique pollutant because it can be metabolized into highly toxic compounds. There have been some well documented instances of mercury pollution. Some of them include the high concentration of mercury in many species of plants, fish, shellfish from many parts of the world (Bargagli and Barghiani 1991; Giordano et al. 1991; Mason 1990; Renzoni 1990; Sapunar et al. 1990; Thompson and Furness 1990), the occurrence of Minamata disease in Japan in 1956 and in 1965 (Mason 1990; Harrison 1990), the contamination of seeds in Sweden in 1960 causing a decrease in the fertility of birds (Ui 1971), the bioaccumulative capability for mercury of barracuda and shark in Lake Murray, Papua New Guinea (Kyle 1981; Peter 1978) and of shrimps and worms in a Hawaiian estuary (Luoma 1974). Several researchers have been conducted in the Philippines to determine the mercury levels and/or their effects in various samples (Balagot et al. 1987; Luis et al. 1987; Panganiban et al. 1987; Rosell 1985; P.S. Kapauan et al. 1982; Del Callar et al. 1975).

The major uptake route for many aquatic organisms is directly from the water, so that to a certain extent, mercury concentrations in the tissues reflect concentrations in water. Carnivores at the top of the food chain such as birds and mammals including humans, obtain most of their pollutant burden from aquatic ecosystems by way of their food especially fish (Mason 1990). People who eat contaminated fish regularly, therefore, are most exposed to the risk of chronic poisoning.

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[1] Supported by Science Foundation, University Research Coordination Office, De La Salle University

In as much as the residents of Taguig rely mostly on fish coming from Laguna de Bay (Taguig and Binangonan area) the researcher thought of analyzing the mercury content of selected fish like dalag *Ophicephalus striatus Bloch* , kanduli *Arius thalassinus Ruppell*, bia *Amblygobius phalaena Curvier et al. Valenciennes* and tilapia *Tilapia nilotica Linnaeus* to determine the degree of mercury contamination of Laguna de Bay and to evaluate metal concentration in fish muscle tissue collected during the peak of dry (April) and wet (October) seasons.

Dalag is capable of living in slightly brackish water. This species has been stocked in lakes up to an elevation of 1,000 m above the sea level. It is present as predator in fresh water carp and tilapia ponds and in ricefields as extraneous fish. This fish is capable of living in foul water by virtue of its accessory respiratory labyrinthine organ and can survive for months without water when búried in moist soil. Its larvae and young fry feed on algae and protozoans. The young take small crustaceans along with restricted portion of vegetable matter while the adults are highly carnivorous and dreaded as predators of other pond fish. The species reaches maturity in about two years. Parent fish prepare nest by biting off vegetation over a circular area in shallow water on the edge of the pond. Eggs are laid in a thin layer and carefully guarded by the male fish. Larvae hatch out within three days and attain adult habits when about nine weeks old (Conlu 1986).

Kanduli is a bottom-dweller of marine and estuarine waters and tidal rivers. It is classified as carnivorous feeding on crustaceans, molluscs, other invertebrates and small fish like *Pelates plumbes* and species of the Family Gobiidae. From January to March it feeds mostly on pelagic fish and from April to June it feeds on demersal forms like snails. It is oviparous and the eggs are orally incubated by the males. It gathers in schools during breeding season, becomes pelagic, then migrates to the bottom for spawning. It spawns during summer (February to May) in Laguna de Bay (Conlu 1986).

Bia is a small-sized, bottom fish inhabiting bays and occurs also in the sea. It feeds on crustaceans and worms. It is an oviparous species (Conlu 1986).

Tilapia is euryhaline, thriving and reproducing equally well in fresh and brackish waters up to 30 ppt salinity. It thrives at an optimum temperature between 20-30°C. Classified as an omnivore, it feeds on plankton, aquatic plants and artificial food like rice bran, soya, bread crumbs, boiled and chopped kangkong *Ipomoea aquatica* Forsskal. The young feed on diatoms, green algae and small crustaceans; the adults feed equally on plankton, filamentous green algae, decomposing vegetable matter and supplementary feeds like rice, soya, cocoa, various flours and oil cakes. This species digs a six cm deep trench on the bottom when spawning. The male requires considerable territory guarding and accosting each intruder with hostile behaviour. The female is a mouthbrooder with a regular region when spawning. It breeds throughout the year and the annual frequency of spawning varies with the climate and food. Spawning takes place during the warm season. The frequency of spawning is from six to eleven with an interval of 22 to 40 days (Conlu 1986).

MATERIALS AND METHODS

Laguna de Bay is the largest lake in the Philippines with an area of 90,000 ha. Its greatest length from E to W is 25 mi and its greatest breadth N to S is 21 mi (Foreman

1985). It is supplied by small streams coming from the mountainous areas around it. The shores of this lake are flooded during wet season and are covered with aquatic plants like water hyacinth *Eichornia cresssipes* (Mart) Solm. These floods recede as the dry season approaches, but only partially so from the south coast, which is gradually being incorporated into the lake bed. It serves as a medium for easy and cheap transportation. It is considered as a good source of fishery production which helps the economy of the people. Fresh water fish like dalag, kanduli, bia, and tilapia abound in the lake between Taguig and Binangonan area (Fig. 1). It furnishes Taguig and nearby markets with choice fish that command higher price.

Collection and Transport of Samples

The live dalag, kanduli, bia and tilapia were taken from the area through the help of fishermen for twelve months, starting from August 1989 and ending in July 1990. The fish samples placed in a Styrofoam box plus the water samples kept in polyethylene liter bottles were transported to the testing laboratory in Bicutan, Taguig, Metro Manila. One kilogram of each kind of experimental fish measuring 15 -20 cm long were cleaned first before analysis was done. Sample preparation was based on the procedure specified by the Perkin Elmer mercury analyzer manual (Thorpe 1971; Uthe et al. 1970).

Laboratory Work for Mercury Determination

Sample Preparation

From one kilogram of homogenised muscle tissue per type of fish, 1.0 g pulverized sample per replicate was weighed. Four ml of concentrated sulfuric acid was added to the sample in a beaker which was immersed in a larger beaker of cold water to avoid overheating. The vessel was then covered with plastic film. The sample was dissolved in a shaking water bath at 50°C until a clear red brown solution was obtained after 2-3 hr. The samples were cooled in ice and 15 ml of 6% potassium permanganate was added from a buret while swirling the samples in the ice bath. They were allowed to stand overnight at room temperature. Two ml of 20% hydroxylamine hydrochloride solution was added slowly while swirling. They were allowed to cool and to stand for 2 min. The samples were covered and shaken to remove the gas. The volume of the samples was adjusted to 100 ml by adding deionized distilled water and transferred to a BOD bottle. The same procedure was followed in preparing the water sample for analysis.

Analysis

One ml of 20% stannous chloride solution was added to the sample and was covered immediately with plastic film. The cap was screwed tightly and the beaker was swirled. After swirling, the samples were allowed to stand for 10 min. They, they are shaken vigorously. The aerator was inserted and the mercury content of the solution was read in ug/ 100 ml for the three replicates. The resulting digest were analysed for total mercury together with appropriate blank and standard solutions using atomic absorption spectrometry. The concentration of mercury per fish sample was then computed in ug/ g. All the analysis were done in triplicate. The figure reported is the mean of the three replicate.

RESULTS AND DISCUSSION

Variations Among Fish Species

Based on the data presented in Table 1 and Fig. 2, dalag shows the highest value of total mercury (0.021 ug/g) while tilapia shows the lowest (0.008 ug/g). In a decreasing order of concentration, the metal muscle tissue distribution is as follows: dalag (0.021) > kanduli (0.020) > bia (0.013) > tilapia (0.008).

Analysis using two-way ANOVA (Table 2) showed a significant difference in the mean total mercury content in ug/g in the different fish samples, among the different months and the interaction between these two variables.

A significant difference using Duncan's Multiple Range test (indicated by different letters in Table 1) and T-test (Table 3) was also found between means of total mercury in dalag and in bia (0.00774), dalag and tilapia (0.01246), kanduli and bia (0.00708), kanduli and tilapia (0.01180), bia and dalag (0.00774), bia and kanduli (0.00708), bia and tilapia (0.00471), tilapia and dalag (0.01246), tilapia and kanduli (0.01180), tilapia and bia (0.00471) at the 0.05 level.

Dalag ranks first in the absorption of mercury since the fish is capable of living in foul water by virtue of its accessory respiratory labyrinthine organ and can survive for months without water when buried in moist soil (Conlu 1986). As it burrows itself in the mud or soil, trace metals in the mud/sediments are absorbed by the gills through a passive process since an optimum concentration gradient is established by absorption of metals on mucus covering the gills and the accumulation of trace amounts in the gill tissues (Connel and Miller 1984). Gills and the intestinal tract are the major uptake sites in fish (Part and Svanberg 1981) while the liver and kidney are the major storage sites of metals (Maker 1986; Young et al. 1986. Although the soft muscle tissues are also sites not spared by heavy metals coming from the gills and other internal organs, studies conducted by Bradley and Morris (1986) and Maker (1986) showed that the lowest concentration of heavy metals were found in the muscle tissue and high concentrations in the gills, gastrointestinal tract, liver and kidney. The close association of benthic fish species with the sediments which generally contain more heavy metals than surface water contribute to the difference in metal loading in their tissues (Madamba and Pamulaklakin 1994).

The difference in total mercury concentration among the fish species can be related to the feeding habits. Dalag is omnivorous and feeds on algae, protozoans, small crustaceans and fish (Conlu 1986) resulting in enhanced accumulation of heavy metals. Bernard and Andreae (1984) reported that the largest metal concentrations were found in omnivorous fish followed by herbivore and carnivore. Mercury not only comes from food but also from the water as fish takes in oxygen. Nutrients are absorbed directly from the water and hence may be expected to reflect the ambient concentration of mercury in the water (Jones 1978).

Seasonal Variation

Data in Table 1 show that seasonal variation influences the concentration of total mercury in the four fish species. Total mercury concentrations in four fish species collected in April (dry season) were significantly higher than in fish samples collected n October (wet season). The values obtained in April samples were 0.0670, 0.0500, 0.0291 and 0.0000 ug/g for dalag., kanduli, bia and tilapia, respectively, while the corresponding values were 0.0102, 0.0081, 0.0086 and 0.0090 ug/g in October samples as shown in Figure 3.

Heavy metal content of aquatic animals originates from two routes of intake: free ions and simple compounds dissolved in the water are taken up directly through the epithelium of the skin, gills and alimentary canal, while others, having been accumulated in food organisms, are incorporated by nutrition (Salanki et al. 1981). Thus, the metal concentration in the water is a significant source of increased metal levels in fish. If a metal is predominantly present in the form that can be readily assimilated by fish or other aquatic organisms, then this could result to a higher metal uptake. Also to be considered is that both dalag and tilapia are bottom dwellers and mercury concentration of the sediment may be expected to be contributory.

Another significant source of increased metal levels in fish is metal-contaminated diet. Plankters which serve as natural food of the fish species have a tendency to accumulate metals in their systems (Madamba and Pamulaklakin, 1994).

In the context of the methods used and in the various other Philippine studies referred to, A.F. Kapauan et al. (1982) pointed out that mercury content of 0.5 ug/g in fish, 0.05 ug/g in sediments and 0.100 ug/g in water may be considered significant and amounts higher than these indicate contamination.

CONCLUSION

Based on the results of this study, the people particularly the residents of Taguig do not face a significant health risk from total mercury. The results show that the levels of the total mercury in the fish samples are below the World Health Organization maximum tolerable consumption of mercury in food of 300 ug or 0.3 mg of total mercury per week (Clark 1989; Harrison 1990; Sapunar et al. 1990).

ACKNOWLEDGEMENT

The author wishes to thank the Science Foundation, University Research Coordination Office (URCO), De La Salle University for providing financial assistance to this project and to Dr. Ernesto Luis and Ms. Josefina Hernandez for the permission to utilize the facililities of the testing center in Bicutan, Taguig, Metro Manila.

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Table 1. Mean Total Mercury (ug/g) of Different Fish Samples for 12 Months.

Fish Samples	Month of Collection												Over Mean Std. Dev.
	Aug. '89	Sept.	Oct.	Nov.	Dec.	Jan. '90	Feb.	Mar.	Apr.	May	Jun.	Jul	
Dalag	0.0103	0.0069	0.0102	0.0120	0.0139	0.0088	0.0063	0.0021	0.0670	0.0670	0.0180	0.0320	0.021A ±0.0023
Kanduli	0.0215	0.0267	0.0081	0.0142	0.0209	0.0060	0.0083	0.0000	0.0500	0.0500	0.0109	0.0092	0.020A ±0.016
Bia	0.0115	0.0119	0.0086	0.0071	0.0144	0.0031	0.0092	0.0000	0.0291	0.0291	0.0141	0.0098	0.013B ±0.009
Tilapia	0.0110	0.0083	0.0090	0.0079	0.0074	0.0062	0.0087	0.0024	0.0000	0.0000	0.0153	0.0113	0.008C ±0.005

Means with the same letter are not significantly different.

Table 2. Two-way ANOVA showing the significant difference in the mean total mercury in the different fish samples, among the different months and the interaction between these two variables.

SOURCE	SUM OF SQUARES	D.F	MEAN SQUARE	F RATIO	PROB.
Fish Samples	4.2802E - 03	3	1.4267E - 03	27.087	7.10E-13
Collecting Mos.	0.016	11	1.4268E - 03	27.088	.000E+00
Interaction	0.013	33	4.0409E - 04	7.672	2.20E-13
Error	5.0565E - 03	96	5.2672E - 05		
Total	0.038	143			p=0.05

Table 3. T-Test (LSD) showing the significant difference between the means of total mercury in fish samples.

FISH COMPARISON	LOWER LIMIT CONFIDENCE	DIFFERENCE BETWEEN MEANS	UPPER LIMIT CONFIDENCE	
1 - 2	-0.00277	0.00066	0.00429	
1 - 3	0.00411	0.00774	0.01137	***
1 - 4	0.00873	0.01246	0.01618	***
2 - 1	-0.00429	-0.00066	0.00297	
2 - 3	0.00337	0.00708	0.01079	***
2 - 4	0.00800	0.01180	0.01560	***
3 - 1	-0.01137	-0.00774	-0.00411	***
3 - 2	-0.01079	-0.00708	-0.00337	***
3 - 4	0.00092	0.00471	0.00851	***
4 - 1	-0.01618	-0.01246	-0.00837	***
4 - 2	-0.01560	-0.01180	-0.00800	***
4 - 3	-0.00851	-0.00471	0.00092	***

Legend: Alpha = 0.05 Confidence = 0.95 df - 88 MSE - 0.0000567
1. Dalag Critical Value of T = 1.98729
2. Kanduli Comparison significant at the 0.05 level are indicated by ***
3. Bia
4. Tilapia

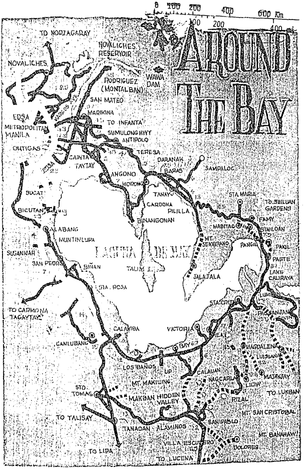

Figure 1. Map of Laguna de Bay Showing Bicutan, Taguig and Binangonan Areas.

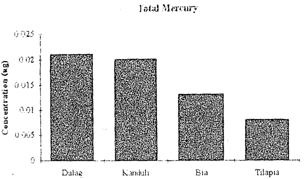

Figure 2. Comparison of total mercury levels in different fish species.

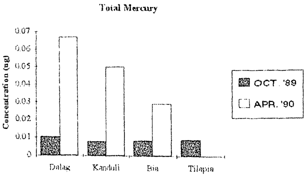

Figure 3. Seasonal variation in total Hg contents of four fish species.

PERFORMANCE OF GMELINA ARBOREA WITH VAM INOCULATION IN ACID SOIL

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ABSTRACT

A field experiment to determine the effects of VAM inoculation on the growth and survival of *gmelina* *in acid soil was conducted in Villa, Baybay, Leyte from November, 1992 to June, 1993. A randomized complete block design with six replications per treatment was used. The treatments were* *gmelina* *seedlings with and without VAM inoculation.*

Survival rate of both the control and VAM treatments were 100 percent. VAM inoculated seedlings were significantly taller than the uninoculated trees. *Gmelina* *inoculated with VAM was significantly bigger and produced higher biomass compared to the control.*

INTRODUCTION

It is estimated that the Philippines has 5.2 M hectares of grassland and open areas which need immediate revegetation (Lamanillo, 1991). These areas are the aftermath of natural occurrence and human activities in the form of shifting cultivation, uncontrolled grazing, forest fires, and blatant logging. The government has been supporting ambitious reforestation programs to bring these marginal open lands into productive forest vegetation. However, reforestation activities are costly endeavors, and considering their meager financial allocations as against the vast hectarage of areas needing revegetation, novel forestation strategies should be explored.

Recently, it was claimed that 6.5 M hectares of forest lands are badly denuded and needed immediate rehabilitation. The Department of Environment and Natural Resources (DENR) reported that the rate of forest degradation is 199,000 hectares annually (Foronda, 1991). In response to the situation, the government targeted these areas and aimed to reforest at least 100,000 hectares annually. In early 1990, the National Forestation Program (NFP) reported that 131,000 hectares were planted in 1989 with an average survival rate of 77% (Foronda, 1991).

The active participation of various groups, motivated by the expected financial return from the forestation programs, will enable the NFP to achieve its proposed goals. However, continuous innovation in technology is required for the reforestation strategies to become cost-effective. One promising approach is the integration of fast-growing trees with VAM inoculation.

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METHODOLOGY

A field experiment on the VAM inoculation to *Gmelina* seedlings were done in Villa, Baybay, Leyte from November, 1992 to June, 1993.

The VAM inoculant containing the organism *Glomus macrocarpum* was secured from the Institute of Biotechnology (BIOTECH), UPLB, Philippines. As previously assessed by the staff of the mycorhiza laboratory at BIOTECH, this inoculant contained about 100 spores per gram of soil carrier. Application of the VAM into the seedling was done right after root initiation of Gmelina. This is to ensure that no other fungi would infect the newly established roots. One to two grams of VAM inoculant were placed in the prepared polyethylene bags containing the potting medium. Newly germinated *Gmelina* seedlings were then transferred into each bag making sure that the inoculants were one to two inches below the newly established roots. The seedlings were kept in the nursery until use (about one month).

A randomized complete block design with six replications per treatment was employed. The treatments were *Gmelina* seedlings with and without VAM inoculation.

One month old *Gmelina* seedlings with the inoculants were translated in the prepared contour lines at a distance of four meters between hedgerows and one meter between seedlings. The newly established hedgerows were cleared of weeds by handweeding throughout the duration of the experiment. Chemical analyses of the soil were done before and after the termination of the study.

RESULTS AND DISCUSSION

Survival rates of both the control and VAM treatments were 100 percent. Careful raising of *Gmelina* seedling from germination to hardening and extra care in the transport of the seedlings from the nursery to the experimental area were employed, hence, the 100 percent survival.

The monthly plant height of *Gmelina* with and without VAM inoculation is presented in Figure 1. *Gmelina* inoculated with VAM was significantly taller compared to that of the uninoculated seedlings one month after establishment. In the second month, no significant difference was noted between the two treatments. Significant differences in height of *Gmelina* were again observed during the third, fourth, fifth and sixth months after establishment. VAM inoculated seedlings were significantly taller than the uninoculated ones.

The stem diameter of *Gmelina* at termination was also recorded. It could be noted that Gmelina inoculated with VAM was significantly bigger compared to the control. Gmelina treated with VAM could have efficiently absorbed soil nutrients and water, hence, the bigger stem diameter.

Figure 2 presents the biomass of *Gmelina* inoculated with VAM on dry weight basis. Gmelina inoculated with VAM produced significantly higher biomass, regardless of the plant parts measured. The rapid growth of the VAM treated seedlings could be due to the increased surface area of roots making them efficient for water and nutrient absorption. Hence, higher biomass were produced. Moreover, the applied inoculant could have effectively infected the secondary roots of *Gmelina* thereby promoting nutrient uptake and enhancing growth.

VAM application did not significantly affect the pH, organic matter, nitrogen, and phosphorus content of the soil. Theoretically, VAM increased the availability of nutrients, particularly P, to plants due to the release of phosphatase from the fungi (Turnbull and De La Cruz, 1990). The rapid increase of these organisms would, therefore, accumulate higher amount of the phosphatase in the soil for plant growth. However, the mycorrhizal association takes some time to develop before its effect can be manifested in the plant and in the soil. In this experiment, it is evident that the effects of VAM have only started to be exhibited in the enhanced growth of the plant. It is then possible that effects on the soil may be manifested later when the nutrients contained in the biomass will be returned to the soil via litterfall in significant proportions as the tree matures.

IMPLICATIONS

The Philippine government has embarked on numerous reforestation programs to bring much of the marginal grasslands into forest cover. Unfortunately, the soils of these grasslands are very infertile, with low amounts of nitrogen, phosphorus and some micronutrients. Thus, survival and growth of reforestation species are very poor.

The government has recently launched reforestation activities through contract programs by NGOs and interested individuals who have the capability to plant and manage newly planted trees. Therefore, in support to the government efforts to accelerate reforestation program, all possible and effective forestation strategies should be explored and developed. One alternative approach is by the involvement of upland farmers through smallhold forestry.

This study had shown that an upland farm in Villa, Baybay, Leyte could be initiated to have a good *Gmelina* forest cover in 6 months time with VAM inoculation. Therefore, planting *Gmelina* in upland farm would promote higher added farm income for the household.

CONCLUSION AND RECOMMENDATIONS

Based on the results obtained it could be concluded that inoculating Gmelina seedlings with VAM before planting results to earlier seedling growth and establishment.

If similar experiment will be conducted, a longer time of observation is recommended to allow sufficient time for the symbiosis between the fungi and roots of the plants to develop.

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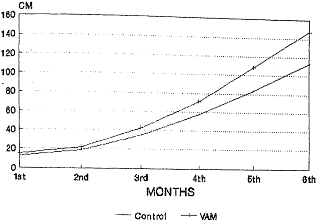

Figure 1. Monthly height of *gmelina.*

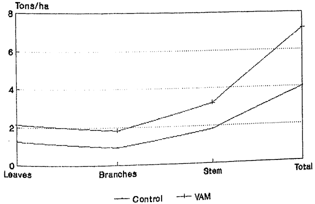

Figure 2. Dry biomass of *gmelina* with VAM.

INDEX TO THE PHILIPPINE JOURNAL OF SCIENCE
VOLUME 125, Nos. 1-4
(January-December, 1996)

A

acid soil, 317
Adrenocortical gonadal, 237
    "parallel type", 237
    "inverse type", 237
Agarwal, Sharmila Dasadhikari, Subho Ghosh, Santasri Sengupta, Sajukta Sarkar and Asok Ghosh. An Adrenocorticolytic Agent Alters Thyro-Gonadal System in the Pigeon, Columba Livia., 237
Allahabad, 301
Allium cepa L., 259, 261
Allium Cepa L., 279
[*Amblygobius phalaena* (*Cuvier et Valenciennes*)], 305, 306
Ammonium Sulfate, 96, 102
Anemia, 291
Anthropometric assessment, 77
Antifertility agents, 129
anti-inflammatory, 271
antimalarial, 271
antimutagenicity, 271
antitumor, 271-272
Arañez, Adoracion T., and Raquel O. Rubio
Arif, Mohammad and Ahsan A. Vahidy. Toxicity of Fungicides Nimrod and Dithane M-45 on Mitotic Cells of Allium Cepal L., 279
[*Arius thalassinus Ruppell*], 305, 306
arthrities, 271
Ascorbic acid, 292, 296
Atsuete, 259
    Bixin, 259, 263
    Orellin, 259, 263

B

Baccigeroides, 53
Baccigeroides hafeezulai, 53
bia, 305, 306, 307
Binangonan, 305
Biochemical changes, 130
    Protein, 130, 132
    Glycogen, 130, 132
    Acid phosphatase, 130, 132, 133
    Alkaline phosphatase, 131-132
    total cholesterol, 131
    esterrified cholesterol, 131
Bixa Orellana L. 259, 261
Blumlein Configuration 1

C

Camphor, 37, 38, 41
Capsules, 95
Carbonic anhydrase (CA), 243
Carboxylic acids, 37
Chaudhuri, P.S., D.K. Nanda and D. Chaudhuri. Extraction of Octochaetid Earthworms, *Eutyphoeus Gammiei* Using An Aqueous Extract of *Polygonum Hydropiper Linn*, with a Comparison of other Chemical Methods for Estimating Earthworm Populations., 229
"chemical adrenocortectomy", 237, 238
"chemical thyroidectomy", 237, 239
Chilka lagoon, Bay of Bengal, 53
Chrysanthemum, 301-302
Compact pulsed TEA nitrogen laser, 1-2, 9
cough, 271
Cymbopogon citratus (DC) Stapf., 147-149

D

Dalag, 305, 306, 307
De Guzman, Ma. Patrocinio E., A.U., Mondala, A.R., Aguinaldo, C.G., Magsaysay, F.C., Cuaderno, E.V., Castillo, C.M., del Rosario, M.M., Bumanglag, F.N., Bautista, C.C., Umali, E.M., Pamaran, 15
De Guzman, Ma. Patrocinio E., Zenaida V. Narciso, Ruby D. Lara, Michelle S. Adriano, Carmencita G. Magsaysay, Josefina A. Magbitang, and Ma. Regina A. Pedro. An

Assessment of the Nutritional Status of Selected Filipino Urban Elderly., 69
diabetes, 271
didemnid ascidians, 243
dihydroterpene, 161
Diode 3
Dionisio-Sese, Maribel L. Carbonic Anhydrase: Its physiological and Evolutionary Significance in the Marine Symbiont Prochloron., 243
Dithane M-45, 279-280, 284

E

Earthworms, 229
Electrical method, 229
Encapsulation, 95, 102
Environmental pollution, 37
  Municipal waste water, 37
  Industrial effluents, 37
  Sewage, sludge, 37
Essential oil, 147
Ethane, 37, 41
Eucheuma, 157
Eutyphoeus gammiei, 229-230

F

Fellodistomidae, 53
Ferula Jaeschkeana, 129, 131
  "Heeng" (family: Umbellifarae), 129
fever, 271
Fluidized bed method, 95, 100
Food intake, 76
Formalin method, 229-230
Fungicides, 279
Fusaria, 301
Fusaria oxysporum f. sp. 301, 302
FVRice-iron fortified, 291, 292

G

Genotoxicity of Pigment from Seeds of *Bixa Orellana L.* (Atsuete) I. Determined by Allium Test., 259
Gmelina arborea, 317, 318
Gonzal, Dominador G., Loretto U. de la Cruz and Dennis P. Garrity. Performance of *Gmelina* Arborea with VAM Inoculation in Acid Soil., 317
gout, 271
Granulation , 96, 98, 102
Guevara, Amelia P., Andrew Apilado, Hiromu Sakurai, Mutsuo Kozuka and Harukuni Tokuda. Anti-inflamatory, Antimutagenicity and Antitumor Promoting Activities of Mahogany Seeds, Swietenia *Macrophylla (Meliaceae)*., 271

H

Hand Sorting, 229
"hanga", 161
heptane, 161
Hexane extract, 129
high blood pressure, 271
hydrocarbon 161

I

IGR effect, 119-122
Inorganic anions, 37
Ion-chromatography, 37
Iron bioavailability, 291, 292

J

Jones, R. H. Holes in magnetoelectrostatic traps., 59

K

kanduli, 305, 306, 307
kappaphycus alvarezii, 157, 158
  (Doty) Lias, comb. nov., 157, 158
Kappaphycus, 157, 158

L

lactating women, 109
Laguna de Bay (Town), 305, 306
lemongrass, 147-149, 155
Liao, Lawrence M. Validation of Names

Transferred to Kappaphycus Doty from *Eucheuma J. Agardh (Rhodophyta: Solieriaceae*)., 159
lighting purposes, 161
Los Baños, 15

M

Madiaga, Juanita R., Juanita M. Ramos and Leah A. Perlas. Riboflavin Requirements of Some Filipino Lactating Women., 109
Magnetoelectrostatic confinement, 59
Magyani, Ofelia. Separation of inorganic anions and carboxylic acids using sulfonic acids as eluents, 37
mahogany, 271-273
Mancozeb, 279
    Manna, Buddaheb and Indu Bhussan Datta. On *Baccigeroides* Gen. Nov. (*Digenea: Fellodistomatidae*: *Baccigerinae*) in the Intestine of *Setipina* Phasa (*Engraulidae*) at Chilka lagoon of Orissa Coast, India., 53
marine unicellular algae, 243
Meal pattern, 73
meat and meat organs, 112
Megasporangium, 163, 165
Mercury, 305, 309
Methane 37, 41
Microencapsulation, 96, 98, 102
milk and milk products, 112
Models, 31
    Consensus building, 17,31
    Baseline data collection, 17,31
    Training module preparation, 18, 31
    Conduct of training, 19, 31
    Physical/infrastructure development, 22-23, 31
    Cooperative development, 23-24, 31
    Feedback management of violation/sanction, 25, 31
    Setting up of model in schools, 31
    Monitoring and evaluation, 31
Musca domestica L. (PCSIR Strains), 119-122
mutagenicity, 271

N

Nagcarlan, Laguna, Philippines, 147-147
Napthalene, 37, 48, 41
"nature ploughman", 229
Neem extracts, 119-120
Nimrod, 279-280, 284
non-heme iron, 293-294
Nutritional assessment, 70

O

Octochaetid worm, 229-230
Octuene sulfonate, 37, 38, 41
Onion, 259, 261
Onion, 279
[Ophicephalus striatus Block.], 305, 306

P

Permanganate method, 229, 231
"petroleum nut", 161
Photodiode 3
Pittosporum resineferum Hemsl., 161-162, 165
Plasma confinement, 59
Polygonum hydropiper, 229-230
Polyploidy, 280, 285
Polypropylene, 187, 193, 195
Prakash, A.O. and S. Jonathan. Effect of Hexane Extract of Ferula Jaeschkeana on the Reproductive Organs in Cyclic Female Rats., 129
Prochloron, 243-248
Phytic acid, 292, 296

R

rheumatism, 271
rhizosphere, 301
Riboflavin, 109
Rogowski Coil, 1,3
Roma, Roderick L. and Henry J. Ramos. Experimental investigation. and characterization of a compost pulsed TEA molecular laser, 1

S

Salt (Sodium chloride) water method, 229, 231
Salvador-Tolentino, Vivian P. and Priscillano M. Zamora. Development of the Female Gametophyte of *Pittosporum Resimiferum Hemsl.* (*Pittosporaceae*)., 161
San Juan, 15
Setipine phasa, 53
Streefoods, 15
Sulfonic acids, 37
Swietenia macrophylla King, 271

T

Tabassum, R., S.M. Narulain, S.N.H. Naqvi and M.A. Azmi. Toxicity and IGR Effect of Two Neem Extracts on Musca Domestica (PCSIR Strain)., 119
Taguig, 305
Tannic acid, 292, 296
Thermoplastic encapsulation, 185, 189, 195
Thermoset encapsulation, 191, 199, 200
Thyro-gonadal System, 237
tilapia [*Tilapia hilotica Linnaeaus*], 305, 306, 307
Tio, Barbara DJ and Francisco A. Arellano. Polymeric Encapsulation of Heavy Metal Bearing Sludge., 185, 187, 194
Toluene sulfonic as eluents, 37
Torres, Rosalinda C. and Arlene G. Ragadio. Chemical Composition of the Essential Oil of Philippine Cymbopogon Citratus (DC) Stapf., 149
Toxicity, 119
Toxicity, 279

V

Valdez, Divinagracia A., Aida C. Mallillin, Erlinda M. Cruz, Miriam D. Kuizon and Ma. Isabel Z. Cabrera. Iron Absorption from FVRice-Iron Fortified., 291
VAM inoculation, 317, 318
Villa, Baybay, Leyte, 317, 319
Vitamin, 109

W

Wasterwater treatment, 185
Water pollution, 37

Y

Yokota, Yuji, Josie L. Pondevida and Cecile F. Alfonso. Preparation of Slow Release Microcapsules Containing Nutrient by Fluidized Bed Coating Methods, 95

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